SECTION 1

PROTOCOL FOR CLINICAL TRIAL AND OBSERVATIONAL STUDY COMPONENTS

1. Summary

1.1 Summary of Clinical Trial

The Clinical Trial (CT) will evaluate the benefits and risks of Hormone Replacement Therapy (HRT), Dietary Modification (DM), and supplementation with calcium/vitamin D (CaD) on the overall health of postmenopausal women. Health will be assessed on the basis of quality of life measurements, cause-specific morbidity and mortality, and total mortality.

Approximately 64,500 women aged 50-79 from 40 centers will be randomized in a partial factorial design and followed for an average of nine years. Women who are eligible and willing to participate in either the HRT or DM components may enter the trial, but eligible participants will be encouraged to enter both components. Women in the HRT component only will be randomized into placebo or estrogen arms if post-hysterectomy, and otherwise to placebo or estrogen plus progestin arms. Women in the DM component only will be randomized into one of two arms (Dietary Change or Usual Diet), and women in both components will be randomized for both. One year after entry, all eligible trial participants will in addition be invited to be randomized further into one of two arms of the CaD component (CaD supplementation or placebo).

It is hypothesized that estrogen replacement therapy (ERT) and combined progestin and estrogen replacement therapy (PERT) will reduce the risk of coronary heart disease (CHD) and of osteoporosis-related fractures. Women who are post-hysterectomy will be randomized to ERT or placebo, while women with a uterus will be randomized to PERT or placebo. Unopposed estrogen is not included as an option for women with a uterus in order to avoid an unacceptable rate of endometrial hyperplasia and consequent change in treatment regimen. The combined regimen (PERT) is not included as an option for hysterectomized women as the progestin is regarded as an unnecessary drug that could diminish some of the hypothesized favorable effects of ERT for such women. The incidence of endometrial cancer and breast cancer will be monitored during and after the trial. The estimated sample size requirement for the primary outcome of (CHD) is 27,500.

Dietary modification in the form of a low-fat eating pattern is hypothesized to reduce the risk of breast cancer, colorectal cancer, and CHD. The estimated sample size requirement for each of the primary outcomes of breast cancer and colorectal cancer is 48,000. The low-fat eating pattern will include reduced intake of total fat and saturated fat, and increased intake of fruits, vegetables and grains.

Calcium/vitamin D supplementation is hypothesized to reduce osteoporosis-related fractures and colorectal cancer. It is estimated that 45,000 women will participate in this part of the trial.

1.2 Summary of Observational Study

The Observational Study (OS) will consist of CT screenees who have participated in at least one visit but are either not eligible or not willing to participate in the trial, and who agree to participate in the OS. It is anticipated that about 100,000 women will be enrolled into the OS, and they will be followed for an average of nine years.

The OS will complement the CT. Data collected at baseline will be related to subsequent clinical events in order to examine the associations of known and putative new risk factors (and protective factors) with disease. Changes in characteristics over the first three years will similarly be related to subsequent clinical events. Serum, plasma, red cell, and buffy coat specimens will be collected and stored for subsequent analysis in cases and controls. The goals of these studies will be to (1) improve risk prediction of CHD, breast cancer, colorectal cancer, fractures, and total mortality in postmenopausal women, (2) create a resource of data and biologic samples which can be used to unearth new risk factors and/or biomarkers for disease, and (3) examine the impact of changes in individual characteristics on disease and total mortality.

2. Background

2.1 General Considerations

The CT/OS is designed to address some of the major causes of morbidity and mortality in postmenopausal women; namely, CHD, breast and colorectal cancers, and osteoporotic fractures. Cardiovascular disease (CVD) is the most common cause of mortality in older U.S. women, accounting for 29-48% of all deaths in the age range 50-79. Coronary heart disease by itself accounts for 13-22% of all deaths in this age range. Both absolute rates and proportional mortalities from these causes increase steeply with age. Among the cancers, breast cancer is the second most common cause of death. It accounts for 4-11% of deaths, and although rates increase with age the proportional mortality from breast cancer is higher at younger ages. Colorectal cancer is the third most common cause of death among the cancers (after breast and lung), and the second most common incident cancer. Rates increase with age and the proportional mortality is steady at about 4%. Death from complications of hip fractures approximate those for breast cancer and colorectal cancer. In addition, fractures account for much morbidity; the annual incidence of fractures increases from 0.5% of women aged 55-64 to 2.3% of women aged 75-84.

Clinical Trial

The goals of the treatments to be tested are to reduce both the morbidity and the mortality associated with the above diseases. Reductions in morbidity from these common diseases should translate into substantial improvements in the quality of life of postmenopausal women and to major societal benefits if the successful treatments are widely adopted by U.S. women. The treatments will also be studied in relation to a range of other diseases and age-related events.

Multiple outcomes will be studied in order to gauge the effect of the proposed interventions, and of the risk factors, on overall health. These include CHD, other cardiovascular diseases, breast and colorectal cancer, other cancers, and osteoporotic fractures. To assess overall benefit and risk for each of the treatments, overall morbidity and mortality, cause-specific morbidity and mortality, and measures of quality of life will be considered. Unresolved issues relating to possible adverse effects such as an increase in breast cancer or endometrial cancer on HRT, and an increase of renal calculi on CaD, will be examined.

Each of the treatments is expected to influence a number of outcomes. Thus, HRT may benefit both CHD and fractures; low-fat dietary pattern may benefit breast and colorectal cancers and also CHD; and CaD supplementation may benefit fractures and also colorectal cancers. The trial has adequate statistical power for each of these outcomes (see *Section 1-A3, Protocol Appendix 3*). In general, the trial does not have statistical power to test subgroup hypotheses; nevertheless, trends in certain subgroups will be of interest. Some of the treatments may have synergistic effects in the subgroups receiving a combination of treatments (e.g., HRT and low-fat dietary pattern on CHD and low-fat dietary pattern and calcium on colorectal cancer), while others may tend to cancel out each other's effects (e.g., HRT and low-fat dietary pattern on breast cancer). Negative interactions may exist, so that the effect of a combination of treatments may be little different from each treatment on its own (e.g., the combination of CaD and HRT may have no greater effect on fractures than either treatment alone). Other subgroup hypotheses are that benefit (or risk) may relate to some baseline characteristic (e.g., the protective effect of HRT on coronary disease may be greater in women with existing CHD, while the risk of breast cancer may be exaggerated in women with a family history of breast cancer).

Observational Study

The OS will be used to improve risk estimates for CVD, cancer, and bone fracture in women, so that high-risk women requiring possible treatment may be more precisely identified. Currently, risk factors in women are poorly quantified, or are unknown. The general approach to be used in the OS will be to use nested case-control or case-cohort analyses of the OS cohort in a variety of applications: to examine the associations of known or putative risk factors (including biomarkers) to disease status at baseline and during follow-up; to find new risk markers using the stored biologic samples and data as a resource; and to examine the association of change in known or putative risk factors on disease outcome.

The OS will provide information on the relationship of personal characteristics such as lipid levels, blood pressure, smoking habits, hormonal status, and dietary habits to future clinical events. The OS will also be used to identify new risk factors. Some of these can be hypothesized a priori, while others may arise later and can then be tested provided appropriate information and/or biological samples have been gathered and stored at baseline. Biomarkers of disease in the form of protein polymorphisms and DNA markers are increasingly being identified. The OS will also be used to examine the impact of involuntary change (i.e., change not induced by treatment) in risk factors on disease outcomes. For example, there is great interest currently in the phenomenon of excess mortality from a variety of causes in persons with low levels of blood cholesterol, albumin, and body weight. The OS will provide an opportunity to test the hypothesis that low levels of blood cholesterol are associated with mortality through the presence of underlying debility or disease which caused both a decline in previously higher levels, and subsequent mortality.

2.2 Hormone Replacement Therapy

2.2.1 Hormone Replacement Therapy and Coronary Heart Disease

The Magnitude of the Problem

The incidence of CHD increases substantially in the decades following the menopause. Both the rates and the proportion of all deaths from CHD increase with age. In 1988 the CHD mortality rates/100,000 (and percentage of all deaths) for U.S. women of ages 50-59, 60-69, and 70-79 respectively were 76 (13%), 260 (19%), and 718 (22%). Coronary heart disease is the leading specific cause of death for women and accounts for the deaths of about 250,000 women each year (National Center for Health Statistics, 1990). CHD in women generally occurs 10-12 years later in life than in men, but because rates approach those of men in the older ages, and there are more older women than older men, overall about half of all coronary deaths occur in women. Almost all these deaths occur in postmenopausal women.

The Potential Role of HRT

The decrease in the circulating levels of estrogens following the menopause is thought to contribute to the increased rates of CHD (Barrett-Connor and Bush, 1991; Korenman, 1990; Godsland et al., 1987). In premenopausal women estrogens may retard the development of atherosclerosis and protect against CHD through their favorable effects on lipoprotein metabolism (and possibly on nonlipid factors such as fibrinogen, blood pressure, insulin levels, body fat distribution, and direct effects on the arterial wall). Reduction in estrogen levels may account in part for the observation that low-density lipoprotein (LDL)-cholesterol levels increase during the transition into the menopause, and continue to increase for some 10-15 years thereafter. There is also a modest decrease in HDL-cholesterol levels during the menopause (Matthews et al., 1989). The effects of exogenous estrogens are pronounced: ERT decreases LDL-cholesterol levels by about 15% and increases HDL-cholesterol levels by a similar amount (Miller et al., 1991; Rijpkema et al., 1990; Walsh et al., 1991). The Postmenopausal Estrogen/Progestin Interventions (PEPI) trial found that women aged 45-64 years randomized to ERT and various PERT regimes experienced similar significant decreases in serum total and LDL cholesterol concentrations, and significant increases in serum HDL cholesterol concentrations, compared with women randomized to placebo. The improvement in cholesterol profile was most marked in the ERT arm, and with PERT arms also giving important, but somewhat variable, improvements.

Some 32 studies have examined the relationship between exogenous estrogen use and CHD. A significantly reduced risk of CHD for women taking ERT has been reported in 11 of 15 published cohort studies and in each of three published cross-sectional angiographic studies (summarized in reviews by Bush et al., 1987; Stampfer et al., 1991; Grady et al., 1992). An additional 13 case-control studies reported less consistent results, and the single small clinical trial yielded promising but inconclusive results (Nachtigall et al., 1979). Various meta-analyses of the pooled studies have indicated highly significant average risk reductions for CHD of 35% to 45% (Bush et al., 1987; Stampfer et al., 1991; Grady et al., 1992), while risk reduction for the combined internally controlled prospective (n=12) and cross-sectional angiographic (n=3) studies was even higher at 50% (Stampfer et al., 1991). In some studies, risk reductions were observed for non-fatal as well as fatal CHD and other CVD, and for all-cause mortality. At least three studies have reported that the risk reductions appear to be even more substantial in women with existing vascular disease (Bush et al., 1987;

Sullivan et al., 1990; Henderson et al., 1991). The data on stroke are less consistent than that for CHD; combined fatal and nonfatal strokes appear not to be reduced, though the studies that provided separate data for fatal stroke consistently showed a decrease in ERT users (Grady et al., 1992). The benefits of HRT appear to increase with prolonged use and current use compared to previous use, though the data are scanty. It is not known whether obese women, who tend to have higher levels of endogenous estrogen, will have the same (hypothesized) benefits from HRT as do lean women.

Progestins are thought to counteract some of the physiologic effects of estrogens. However, it is not known whether progestins will also counteract the putative clinical benefits of estrogens. Cross-sectional studies of CHD risk factors (Nabulski, 1993) and clinical trials (Rijpkema, 1990; Miller, 1991; Lobo, 1994; PEPI, 1995) have provided mixed results. The LDL cholesterol lowering effects of ERT and PERT appear to be equivalent, but in the trials PERT induced a lesser increase of HDL cholesterol than ERT. PERT also induced increases in post-challenge blood glucose levels (but not insulin levels) (Lobo, 1994; PEPI, 1995). However, triglyceride and Factor VII levels were lower on PERT than ERT (Nabulski, 1993; Lobo, 1994). Studies in non-human primates suggest that the anti-atherogenic effects of ERT and PERT are similar, and are mediated through direct vascular effects and LDL cholesterol rather than HDL cholesterol (Adams, 1990; Kushwaha, 1991). Epidemiologic studies (Thomson, 1989; Rosenberg, 1993; Psaty, 1994; Falkeborn, 1992) suggest that the risk reduction for CHD will be similar for users of ERT and PERT. These limited data have not been confirmed in clinical outcome trials.

It is also not clear whether the apparent benefits of HRT from these observational data are largely due to a process of self-selection by which healthier individuals are prescribed HRT, or by other selection biases in the inclusion of subjects or reporting of study results. Such biases may not only exaggerate the apparent benefit, but may also underestimate the magnitude of adverse effects. Studies that have attempted to control for confounders have generally concluded that HRT exerts an independent effect (Stampfer et al., 1991; Henderson et al., 1991; Bush et al., 1987); however, it is almost impossible to control adequately for these (and other, possibly unrecognized) sources of bias in observational studies. Therefore, although the observational studies provide a basis for developing a hypothesis that HRT may reduce the risk of CHD, such a hypothesis can only be tested reliably by a large, well-designed randomized trial.

2.2.2 Hormone Replacement Therapy and Fractures

The Magnitude of the Problem

While fractures are not a major overall cause of death, those women who are hospitalized for hip fracture have a mortality rate as high as 30% from complications such as thromboembolism, fat embolism, pneumonia, and surgical deaths. Fractures are common at older ages and are a major cause of morbidity and loss of mobility (Black et al., 1992 a, b). A woman aged 50 has been estimated to have a 15% chance of being hospitalized for hip fracture during her remaining lifetime (Black et al., 1992 a, b). Annual fracture rates increase markedly with age, being negligible at ages below 55, but rising to 0.5%, 1%, and 2.3% in the age groups of 55-64, 65-74, and 75-84, respectively (Melton et al., 1987). For hip fractures the corresponding rates are 0.1%, 0.3%, and 1.2%. At any age the rates in women are about twice as high as those in men (Melton, 1990). Vertebral fractures are more common than hip fractures but are not usually associated with increased mortality. Other fractures which are associated with osteoporosis include fractures of the pelvis, distal forearm, and proximal humerus.

The Potential Role of HRT

In the main, fractures result from the interplay between bone mass and trauma (Grisso et al., 1991; Melton, 1990). Severe trauma may cause fractures irrespective of bone mass, while even daily activities may result in fracture when bone mass has been severely depleted. Bone mineral density is a particularly good predictor of fractures of the hip, spine, and radius (Black et al., 1992 a, b). Bone loss with aging occurs because the rate of bone formation does not keep pace with the rate of bone resorption. Postmenopausal women lose about a third of their cortical bone and one-half of their trabecular bone. Risk factors relating to bone loss include female sex, increasing age, Caucasian race, oophorectomy, chronic use of oral corticosteroids, early menopause,

prolonged immobility, and insufficient dietary calcium. Protective factors include ERT, obesity, and physical activity (Melton, 1990).

Estrogen status is a particularly important determinant of bone mass. Women have an accelerated bone loss at a rate of about 3% per annum immediately following the menopause which is thought to be related to decreases in estrogen levels. Thereafter, bone loss with aging continues at a slower rate of about 1% per annum (Bilezekian et al., 1992; Steiger et al., 1992). Estrogens can prevent both these losses through preventing bone resorption but may be unable to actually increase bone mass (Bilezekian et al., 1992; Prince et al., 1991). Progestins may also aid the maintenance of bone mass. The major effect of estrogens on bone mass is in the years immediately following the menopause, while the peak rate of fractures occurs some decades later. Nevertheless, at any age estrogens may have the potential to prevent further loss of bone, suggesting that even at advanced ages women receiving estrogens may benefit compared to those who do not. Observational studies indicate that women taking estrogens do have greater bone mass and a lower fracture rate (Johnston et al., 1991). However, the effectiveness of estrogens in preventing fractures has not been adequately tested in a clinical trial, due to the large numbers of women needed to obtain a definitive result.

2.2.3 Potential Adverse Effects of Hormone Replacement Therapy

The use of estrogen increases the risk of endometrial cancer, and may increase the risk of breast cancer and of thromboembolism (Barrett-Connor, 1989; Colditz et al., 1990; Willett, 1989; Goldman et al., 1991; Whitehead et al., 1990).

Analyses of pooled observational data have yielded conflicting results in regard to the risk of breast cancer. Sources of bias exist in the observational data, and it is difficult to predict what their effect is on the findings. For example, closer monitoring of patients on HRT may result in more cancers being identified than in the control group, leading to an overestimate of the risk. On the other hand, doctors may be reluctant to prescribe HRT to high-risk women, which may lead to an underestimate of the risk. Meta-analyses indicate that the overall risk of breast cancer from estrogen appears to be increased by a nonsignificant 7% among users of estrogen replacement (Dupont et al., 1991). Risk appears to be related to duration of estrogen use (increasing by 20% after 10 years, and 30% after 15 years), timing (higher in premenopausal women), dose (higher at doses of conjugated equine estrogens above 1.25 mg/day), type of estrogen (higher for estradiol than conjugated equine estrogens), and family history of breast cancer (risk twice as high in women with a family history) (Steinberg et al., 1991). Importantly, there is no consistent evidence that conjugated equine estrogens at a dose of 0.625 mg/day is associated with significantly increased risk.

The relative risk for endometrial cancer incidence appears to be about 1.5 overall but may be as large as 4- to 10-fold over six years of estrogen treatment, and may persist for some years following cessation of treatment (Whitehead et al., 1990). However, the risk of death from endometrial cancer apparently is not increased. This may be because the endometrial cancers are identified early in these women who are generally under close surveillance, or because the type of endometrial cancer induced by estrogen therapy is relatively non-invasive. It is not known whether the risk for endometrial cancer is reduced by regular monitoring of the endometrium, or by early treatment of endometrial hyperplasia. The expected high incidence of endometrial hyperplasia with unopposed estrogen (e.g., PEPI, 1995) and the consequent need for treatment with progestin precludes the study of long-term use of unopposed estrogen in women with a uterus.

The addition of a progestin may reduce or eliminate the risk of endometrial cancer (Whitehead et al., 1990), while the effect of progestins on breast cancer risk is uncertain. In practice, physicians are increasingly adding progestins to ERT in women who have an intact uterus. Progestins tend to reduce the increase in HDL-cholesterol engendered by estrogens, but appear not to influence LDL-cholesterol (Kushwaha et al., 1991; Miller et al., 1991; Rijpkema et al., 1990; PEPI, 1995). It is not known whether the addition of a progestin will counteract the potential benefit of ERT on vascular disease. Progestins may increase the incidence of physical side-effects such as breast tenderness, bloating, edema, withdrawal bleeding, and abdominal cramping, and they also may increase the incidence of psychological side effects such as anxiety, irritability, and depression. The side effects appear to be dose-related, and to be less frequent at smaller doses.

Since CHD accounts for a far larger proportion of all deaths in postmenopausal women than cancers of the breast or endometrium combined, a reduction of 25-50% in CHD will outweigh even substantial increases in deaths from these cancers (Goldman et al., 1991). For example, at age 70-79 CHD accounted for 22% of all deaths in 1988, while cancer of the breast accounted for 4% and cancer of the endometrium for 1% (National Center on Health Statistics, 1990). ERT has also been reported to increase the risk of thromboembolic events. However, thromboembolism accounted for only a small proportion of deaths (1%) in postmenopausal women.

2.2.4 The Need for a Controlled Trial of Hormone Replacement Therapy

The proposed CT has enormous public health importance, because the diseases to be studied (cardiovascular diseases and fractures) are common, and potential risk reductions obtainable are large. Even if the reductions in CVD mortality and fractures are more modest than those suggested by the observational studies, such reductions could still have a major public health impact, provided that they are not offset by substantial increases in deaths from breast cancer, endometrial cancer, or thromboembolic events.

Currently, a large proportion of physicians do not prescribe postmenopausal HRT beyond the few years after the menopause, either because they are not convinced that such therapy is effective, or because they are concerned about adverse effects. The unfavorable experience of men with preexisting coronary disease who were prescribed large doses of estrogens in the Coronary Drug Project (CDP) have raised doubts about the advisability of using estrogens in patients with CHD, even though the doses currently in use are much smaller than those used in the CDP and the effects in women may be quite different from those in men (Canner et al., 1986; Byar et al., 1988).

When they do prescribe estrogens to postmenopausal women for the purpose of reducing CVD risk, physicians are unsure as to whether the estrogens should be accompanied by a progestin. Though progestins are commonly prescribed together with estrogens in women with a uterus (to protect against endometrial cancer), the epidemiologic data for PERT suggesting benefit in regard to CVD is limited. Progestin-estrogen replacement therapy partly reverses the metabolic effects of ERT, and thus may not have the same magnitude of effect on CVD as ERT. Because of these uncertainties, it is likely that large numbers of women who may benefit from HRT are not receiving it.

These doubts are unlikely to be resolved by further observational studies, because observational studies cannot adequately control for confounding due to differences in the characteristics of women who are treated with HRT compared to those who are not. The similarity of the risk reductions for CVD, CHD, and for all-cause mortality suggests that some or all of the apparent benefit associated with estrogen use may be due to confounding. Some of the possible confounding influences arise from the self-selection of women who go onto hormones, selection bias by physicians as to whom they prescribe hormones to, and socioeconomic biases. A clinical trial, in which selection bias is eliminated by random allocation to treatment and control groups, is needed to evaluate the true benefit of estrogen, and of estrogen plus progestin. Such a trial will provide critical guidance as to the indications for HRT for conditions other than the relief of postmenopausal symptoms. In order to provide this guidance, the trial should test both ERT among hysterectomized women and PERT among women with a uterus.

The CT will be able to assess the benefits and risks of HRT, and thereby provide information on the global impact on women's health. The CT will evaluate the benefits and risks of HRT on CHD, cancers of the breast and endometrium, fracture rates (in particular, hip fractures), quality of life, and total mortality. In addition, information on the possible mechanisms (such as plasma lipids, clotting factors, blood pressure, plasma insulin, body fat distribution) through which estrogens mediate their putative protective effect on CHD will be obtained and analyzed during the trial.

2.3 Dietary Modification

2.3.1 Dietary Modification and Breast Cancer

The Magnitude of the Problem

Among U.S. women, breast cancer is the cancer with the greatest incidence and the one with the second greatest mortality after lung cancer (National Cancer Institute, 1989). In 1991, approximately 175,000 cases of breast cancer were diagnosed and about 44,500 deaths occurred. In 1988 the national mortality rates in the age groups 50-59, 60-69, and 70-79 were 64, 96, and 124 per 100,000 (National Center on Health Statistics, 1990). Breast cancer incidence rates have increased about 1% per year since the early 1970's, whereas mortality rates have remained fairly stable over the past 50 years.

The Potential Role of Diet

International breast cancer incidence rates among postmenopausal women show highly significant positive regression on corresponding per capita dietary fat supply (e.g., Armstrong et al., 1975; Prentice et al., 1990a). In fact, such analysis suggest that a 50% reduction from U.S. fat consumption levels could lead to a two and a half-fold reduction (estimated relative risk of 0.39) in postmenopausal breast cancer incidence (Prentice et al., 1990a). Saturated fat, and particularly polyunsaturated fat, supply correlate with breast cancer incidence in these analyses.

Women migrating from low-fat consumption to high fat consumption areas tend to adopt the higher breast cancer rates of their new country (e.g., Kolonel et al., 1991; McMichael et al., 1988; Margetts et al., 1991). In fact the three-fold higher breast cancer incidence among Japanese women in Hawaii, as compared to Japanese women in Japan (Tominaga et al., 1985) appears to be quite consistent with the international regression analysis noted above upon acknowledging per capita fat supply differences between the two countries (Prentice et al., 1990a).

There is extensive literature relating fat consumption in rodents to mammary tumor incidence (e.g., Carroll et al., 1975). Though these data have been variably interpreted, a recent meta-analysis (Freedman et al., 1990) indicates that dietary fat has a specific positive association with mammary tumor incidence, beyond the association that can be attributed to fat as a source of calories.

Analytic epidemiologic studies have tended to yield equivocal results concerning dietary fat and other dietary factors in relation to postmenopausal breast cancer risk (e.g., Greenwald, 1988; Prentice et al., 1988; Hulka, 1989). In large part, this may be due to a limited range of intakes of fat and other nutrients within populations studied, and to the known major random and systematic errors that attend individual estimates of nutrient intakes based on available dietary assessment techniques.

These factors combine to reduce study power and reliability, and to elevate the importance of minor confounding biases (e.g., Goodwin and Boyd, 1987; Prentice et al., 1989; Byar et al., 1989). Nevertheless, a meta-analysis of the raw data from 12 case-control studies, including several thousand cases and controls, yielded a highly significant positive association between estimated fat consumption and postmenopausal breast cancer risk (Howe et al., 1990). Moreover, the estimated risk relationship appears to be quite consistent with the strong international regression analysis noted above. On the other hand, the three existing sizable cohort studies of dietary fat and breast cancer appear to yield conflicting results. The studies of Howe et al., (1991), and Kushi et al., (1992), report non-significant positive associations that appear to be consistent with the international analyses, while that of Willett et al., (1992), is not suggestive of any positive association between fat consumption and breast cancer risk.

Some of the studies alluded to above have suggested that vegetable intake, or related dietary intakes may be associated with reduced breast cancer risk (e.g., Howe et al., 1990). However, results concerning these associations have often been inconsistent or equivocal in individual analytic epidemiologic studies, quite possibly for the reasons mentioned above.

Feasibility studies of a low-fat eating pattern among healthy women in the age range 45-69 (e.g., Insull et al., 1990; Henderson et al., 1990; Gorbach et al., 1990) show that women randomly assigned to dietary intervention are able to reduce the fat content of their diet to about 20% of calories and to retain the dietary change for two years or more. Change in plasma hormone concentrations were also studied in a subset of women assigned to dietary intervention. These women were found to experience a significant, average 17%, reduction in plasma estradiol concentration following a few weeks of dietary intervention (Prentice et al., 1990 b), thereby adding strength to the low-fat eating pattern and breast cancer prevention hypothesis.

2.3.2 Dietary Modification and Colorectal Cancer

The Magnitude of the Problem

Colorectal cancer is the third leading cause of cancer deaths in U.S. women and the incidence is third only to that of breast and lung cancer (National Cancer Institute, 1989). About 78,500 new cases were diagnosed in 1991 and approximately 31,000 deaths from colorectal cancer occurred. In 1988 national mortality rates for colorectal cancer in the age groups 50-59, 60-69, and 70-79, respectively were 21, 53, and 109 per 100,000 (National Center on Health Statistics, 1990).

The Potential Role of Diet

Epidemiologic and animal studies conducted over the last few decades have established a fairly strong link between dietary factors and colorectal cancer (National Research Council, 1989). Various dietary constituents have been implicated, including fat, excess calories, and reduced dietary fiber. International correlation studies show an approximately linear relationship between total dietary fat availability and colorectal cancer risk (Carroll and Khor, 1975). In fact, such analyses suggest that a 50% reduction from U.S. fat consumption levels could lead to a three-fold reduction in colorectal cancer risk (Prentice et al., 1990a). Studies in migrants from areas with diets low in animal fat and protein to areas with a more typical "Western" diet with high fat intakes show an increase in incidence of colorectal cancer among the migrants when compared to incidence in the country of origin (e.g., migration from Japan to Hawaii, Kolonel et al., 1981; and from Italy to Australia, McMichael and Giles, 1988). A National Cancer Institute sponsored clinical trial is currently assessing the ability of a low-fat, high fiber diet to prevent the recurrence of colonic polyps, which are considered to be precursor lesions for colon cancer.

A rather large number of case-control studies have examined the relationship between estimates of dietary fat consumption, and colorectal cancer risk (e.g., Graham et al., 1988; Jain et al., 1980; Kune et al., 1987; Lyon et al., 1987; Potter et al., 1986; Slattery et al., 1988; Loe et al., 1989; Tuyne et al., 1987, Whittemore et al., 1990). The studies have tended to yield mixed and equivocal results (Kolonel et al., 1987), though collectively they seem to be fairly consistent with projections based on the strong international correlational results (Prentice et al., 1990a). Prospective study results have likewise given mixed results with a study of men of Japanese ancestry (Stemmerman et al., 1984) not suggestive of a relationship between saturated fat and colon cancer, while a recent study in U.S. nurses reported a significant positive association (Willett et al., 1990).

Several international correlation and case-control studies have shown inverse relationships between the intake of high fiber foods and colon cancer risk (National Research Council, 1989; Greenwald et al., 1987). High intake of fruits and vegetables has been fairly consistently related to lower risk of colon cancer, whereas the consumption of cereal grain products has been either unrelated or negatively associated with risk of colon cancer. Analytic epidemiological studies that have had a reasonable capability to assess dietary fiber have tended to suggest a protective effect for fiber consumption (e.g., Trock et al., 1990), while considerable recent interest focuses on the potential of various sources of fiber (e.g., wheat bran versus oat bran) to reduce colorectal cancer risk.

2.3.3 Dietary Modification and Coronary Heart Disease

The Magnitude of the Problem

See *Protocol Section 2.2.1* on Hormone Replacement Therapy.

The Potential Role of Coronary Heart Disease

The etiology of CHD has been linked through international studies to the consumption of high fat diets. Saturated fat intake as a percent of calories correlated strongly (r = 0.84) with CHD mortality rates in the Seven Countries Study (Keys, 1980). A lifelong low-fat diet may in fact exert beneficial effects on CHD rates beyond its influence on blood cholesterol. The slope of the line relating dietary percent calories from saturated fat is nearly two and one-half times greater than that which would be expected if saturated fat operated only by raising serum cholesterol. Migrant studies (e.g., Japanese migrants to Hawaii) suggest an important effect of saturated fat consumption on CHD rates (Robertson et al., 1977). As in the case of cancer, and probably for the same methodologic reasons, it has been difficult to demonstrate a consistent effect of saturated fat on CHD in analytic studies of individuals within populations.

Dietary factors other than saturated fat may influence CHD rates either via reducing blood cholesterol (e.g., food fiber), through decreasing levels of oxidized LDL (by increasing the intake of antioxidants such as selenium, vitamin E, ascorbic acid, and beta-carotene), through effects on the platelet function (fish oils), or through indirect or unknown mechanisms.

Role of Serum Cholesterol on CHD Women

Serum total cholesterol levels generally increase from young adulthood through middle age in both men and women, with levels for men generally higher. However, above age 65, cholesterol and LDL values are considerably higher in women than in men. Increasing levels of serum cholesterol correlate with an increasing incidence of CHD among women up to the age of 65 years. Beyond this age, the association is less robust, but fewer studies are available. There is some evidence that serum triglyceride levels may be predictive of CHD in postmenopausal women. Increasing high-density lipoprotein (HDL)-cholesterol levels appears to be protective in women of any age (Manolio et al., 1992).

Cholesterol Lowering and CHD

The major prospective primary and secondary prevention clinical trials that demonstrate a reduction of CHD events by lowering of plasma cholesterol levels by diet and/or drugs have been conducted in middle-aged men (Lipid Research Clinics, 1984; Frick et al., 1987). Studies in men and women have shown that restriction of dietary fat and cholesterol can lower plasma total and LDL-cholesterol, though the results appear to be somewhat less consistent in women (Ernst et al., 1980; Kris-Etherton et al., 1988). No studies have been conducted in postmenopausal women to determine the long-term effect of a low-fat diet on lipid levels. Furthermore, there have been no large randomized trials in women to study the effects of lowering lipids on CHD incidence. Women's Health Trial feasibility studies (Insull et al., 1990; Henderson et al., 1990) demonstrate a modest but highly significant reduction in plasma cholesterol concentration among women assigned to DM.

2.3.4 The Need for a Controlled Trial of a Low-Fat Eating Pattern

Many types of evidence bear upon the hypotheses of interest in the proposed dietary intervention trial, namely that dietary intakes of fat, grains, fruits and vegetables are related to the incidence of breast and colorectal cancers. Considerable differences of opinion continue to exist among scientists on the "diet-cancer" hypothesis, in large part due to numerous limitations and inconsistencies in the available data.

Animal experiments are important for demonstrating plausible biological mechanisms and for confirming or explaining the results of epidemiological studies, but their results cannot on their own be extrapolated to

humans. If a marker for disease exists, then clinical metabolic studies may be performed to test the effect of DM on the marker. No such marker currently exists for breast or colorectal cancer.

Studies correlating international data on incidence of disease with food disappearance data and migrant studies provide useful information in support of these hypotheses but cannot be entirely relied upon because available dietary data are crude and because results may be subject to confounding and aggregation biases.

Case-control studies overcome some of these problems but suffer from possible biases in the selection of cases and controls and differential recall of dietary intake by cases and controls, as well as from non-differential error in the measurement of dietary intake. Prospective cohort studies avoid selection and recall biases but still rely upon food questionnaires which are known to involve substantial measurement error. These problems are compounded by the narrow range of intakes of the populations typically entering a case-control or cohort study.

Definitive studies to test the effectiveness of dietary interventions to reduce cancer incidence and mortality are not available. The proposed randomized trial of a low-fat dietary pattern, defined as an eating pattern that is low in fat and high in fruit, vegetables and grains, will have an appropriate design and will have the power to provide a definitive answer to a question of great public health importance.

The proposed trial will at the same time provide estimates of the effectiveness of a low-fat dietary pattern in preventing CHD, as well as providing information on the effect of such a dietary pattern on serum cholesterol, blood pressure, and body weight. If a low-fat dietary pattern does reduce the incidence of any one of the clinical outcomes of breast cancer, colorectal cancer, or coronary disease, the public health implications will be important since it can be expected to lead to an even greater emphasis on low-fat dietary patterns and in public health recommendations and in clinical practice. Also, as a result of this CT, dietary guidelines (e.g., National Research Council, 1989) may be able to be refined, and the credibility of such recommendations will be much enhanced.

2.4 Calcium and Vitamin D (CaD) Supplementation

2.4.1 Calcium, Vitamin D and Fractures

The Magnitude of the Problem

See *Protocol Section 2.2.2* on Hormone Replacement Therapy.

The Potential Role of CaD

Insufficient dietary calcium is one of the possible risk factors for osteoporosis and hence for fractures (e.g., Heaney 1982; Cummings et al., 1985; Cummings, 1990). An inadequate intake of calcium is common in women; the NHANES data show that calcium intake in women is 40-50% below that of men, and 75-80% of women have daily intakes below 800 mg, while 25% have intakes below 300 mg. According to the 1984 National Institutes of Health (NIH) Consensus Development Conference on osteoporosis, dietary calcium intake required to prevent negative calcium balance increases from around 1000 mg/day in perimenopausal women to 1500 mg/day after the menopause (NIH Consensus Development Panel, 1984). Intestinal absorption of calcium declines with advancing age (Gallagher et al., 1979). An age-related intestinal resistance to the action of 1,25(OH)2D has been implicated in this impaired absorption (Heaney, 1982), as have age-related changes in parathyroid hormone and 1,25(OH)2D levels (Riggs et al., 1986). Estrogen is known to enhance intestinal calcium absorption and renal calcium conservation (Heaney, 1990). Thus, both estrogen and calcium supplementation can help reverse the negative calcium balance that accompanies aging. On the other hand, low-fat diets are sometimes accompanied by a reduced intake of dairy products and of calcium and may thus increase the negative calcium balance (Holbrook et al., 1991), though reduced calcium intake has not been found in feasibility studies of the DM program to be used in the Women's Health Initiative (WHI) (Insull et al, 1990).

Even though low dietary calcium intake may be a risk factor for osteoporosis and for fractures, the data on the effectiveness of calcium supplements are conflicting (Reid, 1990; Dawson-Hughes et al., 1990; Riis et al., 1987; Prince et al., 1991). This variation may reflect differences in hormonal status and diet of the subjects. In a recent study of older postmenopausal women, calcium supplements were effective in preventing bone loss in those women with a dietary calcium intake of less than 400 mg, but not in those with higher dietary calcium intakes (Dawson-Hughes et al., 1990). The addition of vitamin D appears to increase the effect of supplemental calcium on the prevention of bone loss; it is uncertain whether this is because the absorption of calcium is enhanced, or whether vitamin D exerts an independent effect (Dawson-Hughes et al., 1991). Estrogen therapy reduces bone loss in postmenopausal women, and it is not known whether calcium supplementation in women already on estrogen will induce a significant further reduction in bone loss.

2.4.2 Calcium and Colorectal Cancer

Human observational studies (e.g., Garland et al., 1986; 1989; 1991) and animal experiments suggest that calcium may decrease the risk of colorectal cancer, possibly because increased formation of the calcium salt of bile acids decreases promotion of cancer (Lipkin et al., 1991). Data from controlled trials on the effect of calcium supplementation on colorectal cancer are not available, hence this large trial may provide valuable information.

2.4.3 The Need for a Controlled Trial of CaD

Despite the conflicting data regarding efficacy, many women are currently taking supplements of CaD in the hope of reducing bone loss. Only one trial of the effect of calcium and vitamin D_3 supplementation and fracture rates has been reported; hip fracture rates among healthy elderly women were reduced by 43% (P=0.05) among women completing an 18-month course of 1.2 grams of elemental calcium and 800 IU of vitamin D_3 per day (Chapuy et al., 1992). A definitive clinical trial would provide a rational basis for advising women concerning such supplementation. The CaD component will indicate whether supplementation is effective in reducing bone loss and fracture rates, and in reducing colorectal cancer. Subgroup analyses may provide additional information on aspects such as the effect of varying dietary intake of calcium on efficacy of supplementation in reducing bone loss, the effect of supplementation alone or in combination with estrogens on bone loss, and the effect of calcium alone or in combination with a low-fat dietary pattern on colorectal cancer.

A 1994 NIH Consensus Development Conference panel has recommended that postmenopausal women should have a daily calcium intake of 1000 to 1500 mg to reduce fracture risk, and stressed the need for sufficient vitamin D intake to maximize the benefits of calcium on bone health. The panel also advocated more research on the potential of adequate calcium intake to decrease the risk of cardiovascular disease and colon cancer (NIH Consensus Development Panel, 1994).

2.5 Observational Study

2.5.1 Observational Study Potential

Observational studies have made unique and important contributions to medical knowledge. Historically observational studies have not only generated the hypotheses which were later tested in clinical trials, but have also had a more direct impact on medical practice. For example, a valuable contribution of the Multiple Risk Factor Intervention Trial (MRFIT) was not to be found in the trial results but in the observational data on the large cohort of male screenees. The MRFIT cohort provided very stable estimates of relative and absolute risk for CHD in men by level of serum cholesterol, and these estimates have been of critical importance in the formulation of national guidelines for the prevention of CHD by lowering cholesterol. No comparable data exist for women. Cigarette smoking and lung cancer provide another example of the importance of observational data; the association was so strong and consistent that observational data alone were sufficient to convince health authorities that action to curb smoking was desirable.

Randomized controlled trials offer a unique opportunity to evaluate the influence of preventive measures on health outcomes, since randomization eliminates the possibility that individuals otherwise at altered risk of an

outcome selectively have been exposed to the measure. Nonetheless, we cannot rely on the results of randomized controlled trials for all of our information on the causes of disease and the effectiveness of health interventions. First, some potential causes of disease simply are not amenable to study via randomization. For example, in studies of long-term health outcomes it is rarely possible to randomize individuals to occupational or environmental exposures. Second, because of the costs of large randomized controlled trials, only a limited number of preventive interventions are assessable. In addition, in randomized controlled trials it is necessary to employ a relatively small number of intervention arms, e.g., treated vs. placebo, or treatment A vs. treatment B vs. placebo. Often, the range of potential interventions for a particular health problem is wider than can be encompassed in a single trial. For instance, in the randomized trial portion of the WHI, all women assigned to receive estrogen plus progestin will be asked to take a particular daily preparation at a given dose. Thus, the results from the trial will not speak directly to the influence of other types of progestin, or of hormones taken at different doses or for other durations each month, on the occurrence of breast cancer, myocardial infarction, and other diseases.

Given the foregoing, it is not surprising that many of the inferences made regarding the prevention of disease are based on the results of nonrandomized studies. The latter may take the form of: a) cohort (or follow-up) studies, in which persons with or without (or at various levels of) a given characteristic are monitored for the subsequent occurrence of one or more health outcomes; or b) case-control studies, in which ill and well persons are contrasted for one or more prior exposures. The OS is designed as a cohort study. For efficiency purposes, without the risk of introducing bias, many uses of the OS will involve so-called nested case-control or case-cohort subsampling procedures.

A relative advantage of cohort over case-control studies is the ability to ascertain an individual's exposure status prior to the presence of the outcome, thereby minimizing potential bias that can occur via retrospective ascertainment of exposure. To date, a number of cohort studies have been conducted that have been able, in one way or another, to address risk factors for health outcomes in older women. The planned OS has the potential to provide information that goes well beyond that provided by existing studies. It is large (the Study will seek to recruit some 100,000 subjects) and subjects are to be followed for a relatively long time (9 years). Questionnaires and baseline physical and laboratory examinations will be obtained on all cohort members. In addition, specimens (e.g., samples of blood, separated into its various components) will be obtained and stored for later use. Follow-up of the cohort and ascertainment of illnesses of interest will be highly complete. Finally, the range of health events identified in cohort members will be wide and will encompass the large majority of serious illnesses that occur in middle-aged and older women.

A wide variety of important clinical and public health issues will be assessed with the OS. Firstly, the OS will provide stable estimates of the relative and absolute risks for specific diseases posed by known risk factors such as serum cholesterol (and lipoprotein subfractions), blood pressure, smoking, hormone use, exercise, and obesity for CHD, for example. This information will be gathered by relating information obtained on baseline characteristics to subsequent illness events and mortality. Secondly, the study is designed to address the hypothesis that underlying debility and disease is responsible for the excess mortality at low levels of body weight, cholesterol, and blood pressure. This hypothesis will be tested by relating the markers of clinical and subclinical disease, and change in weight, cholesterol, and blood pressure to subsequent mortality. Previous studies have not had the ability to address this hypothesis because of small numbers, lack of repeated measurements, inadequate ascertainment of subclinical disease, or failure to measure appropriate covariables. The third and perhaps most important purpose is the identification and testing of new hypotheses with regard to disease etiology that are not yet satisfactorily addressed in completed or ongoing studies. In addition to questionnaires and physical data, the gathering of biological specimens at baseline for storage and later analysis will allow hypotheses that arise during the course of the WHI to be examined in nested case-control or case-cohort studies. It is likely that new potential biomarkers of disease, such as protein polymorphisms and DNA markers, will be identified during the course of the WHI. The availability of stored biological material and information on other factors that might confound or modify biomarker-disease relations will facilitate epidemiologic studies of these newly identified potential determinants of disease.

The large size of the overall cohort, combined with the effort that is to be made to include sizable proportions of members of racial/ethnic minorities, will permit for a number of the more common health outcomes the identification of risk factors in individual minority groups. Minority women have not been well represented in

most past or present cohort studies of CVD, cancer, or fractures. The proposed OS can be expected to enroll about 20,000 minority women as subjects. With these much greater numbers, it can begin to explore interracial differences in risk factors for conditions that occur with relatively high frequency, e.g., the major cancers, CVD, hip or forearm fracture, and other age-related outcomes (e.g., diabetes mellitus, glaucoma, urinary incontinence). Similarly it will be possible to explore differences in risk factor impact on other subgroups; for example, those defined by age and socioeconomic characteristics.

For reasons of cost-effectiveness, the OS participants at individual Clinical Centers (CCs) will generally be drawn from a convenient sample rather than a population-based sample. Also, they will be screenees for the CT. The potential loss of "representativeness" will however be mitigated by the wide geographic distribution of the approximately 40 CCs, which will draw on diverse populations, and by the plan to recruit about 20% minority women in the study-wide sample. It is not the intent of the WHI to compare the cohort as a whole with other populations. All the comparisons will be within the cohort itself, e.g., women with and without high blood pressure, and women who do and do not use a progestin to supplement their use of postmenopausal estrogens. Furthermore, many key risk factors for the diseases of interest will be identified in cohort members, so that a relatively unconfounded estimate of the influence of a particular risk factor should be obtainable. Thus, we believe that the generalization of results obtained from the intracohort comparisons will be no less broadly applicable than those of any other epidemiologic study.

The OS capitalizes on the existence of the CT's needs to screen a very large number of potential participants in order to obtain the targeted number of actual participants. Thus, the marginal cost of cohort identification for the OS is exceedingly small - almost all of the screening costs would be incurred even if there were no OS. While the added expense of following a large group of nonrandomized women for health outcomes is substantial, even this expense is considerably smaller than if the human and physical resources were not to be shared with those of the parallel CT.

2.5.2 Need for the Observational Study

There is an urgent need for stable estimates of the magnitude of risk factor impact on health in postmenopausal women; these estimates are not nearly as complete as in men. There is a need for the identification of "new" risk factors, and the cohort design and procedures of the OS allows for exploration of risk factors of uncertain status, or factors which have yet to be identified. There is a need for the elucidation of the mechanisms underlying the excess risk of mortality at low levels of weight, cholesterol, and blood pressure. Finally, there is a need to examine subgroups of women (for example by race, age, SES) in order to determine whether or not the same risk factors operate to the same degree across such subgroups. All of this information is important in setting health policy guidelines. It is unlikely that any of this kind of information will be obtained from clinical trials or from other existing observational studies.

3. Study Objectives

3.1 Objectives of the Clinical Trial

The overall objective of the trial will be to ascertain the benefits and risks of a number of treatments that may improve the health of postmenopausal women ages 50-79. The treatments to be tested are: HRT, low-fat dietary pattern, and supplementation with CaD.

The specific aims of each of these treatments are:

For HRT:

1. To test whether ERT and/or PERT reduce the incidence of CHD and of other CVD.
2. To test whether ERT and/or PERT reduce the incidence of all osteoporosis-related fractures and hip fractures separately.
3. To assess whether ERT and/or PERT increase the risk of breast cancer.

For Dietary Modification:

1. To test whether a low-fat dietary pattern reduces the incidence of breast cancer and colorectal cancer, separately.
2. To test whether a low-fat dietary pattern reduces the incidence of CHD.

For CaD:

1. To test whether supplementation with calcium and vitamin D reduces the incidence of hip fractures.
2. To test whether supplementation with calcium and vitamin D reduces the incidence of colorectal cancer.

Sample size estimates have been based on the first aim for each treatment (see *Section 1-A3, Protocol Appendix 3*), and power calculations have been conducted for the remaining aims. Even though the trial will generally not have sufficient power to test subgroup hypotheses unless there are unexpectedly large effects, various additional analyses will be conducted to obtain information as to whether the effects of treatments appear to vary by participant characteristics or by the presence of another treatment. Subgroup analyses that will be performed will examine:

1. The effect of HRT on the incidence of coronary and other CVD in women with, and in women without, CVD at baseline.
2. The effect of HRT on the incidence of coronary and other CVD and breast cancer in obese and lean women.
3. The effect of supplementation with CaD on fractures and colorectal cancer in women with low, and women with higher, intakes of dietary calcium.
4. The effect of HRT, and of a low-fat dietary pattern, on breast cancer incidence in women at high and at low risk of breast cancer.
5. The effect of HRT plus low-fat dietary pattern on coronary and other CVD and on breast cancer compared to each therapy alone.
6. The effect of HRT plus CaD supplementation on fracture rates, compared to each therapy alone.
7. The effect of HRT on CHD and other CVD among women with a uterus, as compared to hysterectomized women.
8. The effect of HRT, DM and CaD in subgroups of women defined by age and race/ethnicity.

In the HRT, analyses comparing active hormone therapy to placebo, stratified by hysterectomy status, will be conducted to examine the effects of prescribing the hormone preparation most appropriate with regard to a woman's uterine status. This approach also serves to increase power when the effects of ERT and PERT are similar.

The trial will also offer the opportunity to examine certain other questions such as: the effect of each treatment on perceived quality of life, on combined primary and secondary outcomes, and on total mortality; the effects of HRT and DM on lipids, lipoproteins, clotting factors, blood pressure, body mass index, waist-to-hip ratio, and blood glucose; trends in the magnitude of HRT, DM and CaD effects across age categories and across values of other participant characteristics; the relationship to clinical outcomes of (a) baseline biochemical and physical variables, (b) changes in those variables induced by treatment, and (c) adherence. The ability of changes in such intermediate variables to explain an observed relationship between treatment and disease occurrence will also be examined.

The CT will provide valuable information on various other outcomes, even though the study design has not been motivated by considerations of power for such other outcomes. For example, DM will also be studied in relation to other cancers, including ovary and endometrium cancer, and in relation to diabetes mellitus incidence; and CaD supplementation will be studied in relation to cancers other than colorectal, including breast. Importantly, total mortality rates and other summary measures of benefits versus risks will be monitored in relation to each treatment and treatment combination. An important subsidiary aim is to examine the effect of each CT treatment on bone density (see *Protocol Section 8*).

3.2 Objectives of the Observational Study

The overall objective of the OS is to provide information complementary to that obtained from the CT. Measurement of baseline characteristics, remeasurement after three years, storage of frozen blood specimens, and ascertainment of clinical events in a large cohort of postmenopausal women allow the following specific objectives to be formulated:

1. Prediction of risk of outcome on the basis of:
    - Questionnaires and interview data: Women in the OS will be asked to complete the same self-administered questionnaire as CT participants. In addition, they will be asked to complete a supplemental questionnaire at the end of the screening visit at which a woman joins the OS, usually Screening Visit 1 (SV1) and at selected follow-up visits. This will permit the evaluation of associations that cannot be studied in the CT.
    - Physical exam findings: The anthropometric measurements will be related to the occurrence of selected illnesses and mortality.
    - Laboratory data: Some previously studied markers of risk can be examined in considerably greater detail than before, e.g., levels of specific lipid components, whose role in the occurrence of CHD in women is not as well understood as it is in men. Of particular interest will be analyses of stored specimens for recently-developed (or as-yet-to-be-developed) potential biomarkers, e.g., apoprotein subtypes in relation to CHD incidence, or genetic polymorphisms identifiable in stored leukocyte DNA in relation to cancer incidence.

2. Extension of results obtained in the CT to related exposures or regimens: For example, if estrogen use (or calcium supplementation) is found to be effective in achieving a given outcome as measured in the randomized controlled trial, then one could assess whether in the OS a similar relationship is present for that exact regimen, adjusting for confounding variables and exposure durations as necessary. If a similar relationship is found, a relatively high level of credibility could be given to analyses of related regimens outside those studied directly in the randomized trial. For example, if use of estrogens plus a given progestin regimen is found to have a beneficial effect on the incidence of myocardial infarction, the data from the OS can be used to assess the extent to which different progestins/doses/durations can achieve the same effect.

3. Assessment of temporal relationships between risk factors and disease occurrence: Changes in characteristics such as weight or serum albumin or cholesterol levels, or changes in hormone use, could be assessed for their ability to predict rates of selected clinical outcomes. By measuring and controlling for the presence of subclinical disease prior to the change in risk factor status, the ability to infer a causal relation between change in a risk factor and the subsequent incidence of diagnosed disease will be enhanced.

4. Documentation of variation in the incidence of CVD, cancer, osteoporosis and fracture in postmenopausal women on the basis of geographic region and other demographic characteristics, and an evaluation of the extent to which differences among demographic subgroups in the prevalence of identified risk factors account for such variation.

Section 1-A5, Protocol Appendix 5 - Women's Health Initiative Observational Study Overview of Objectives and Hypotheses provides a partial list of risk factors that will be examined in the OS.

4. Study Design

4.1 Overview

The trial will be a partially blinded, controlled clinical trial in postmenopausal women age 50-79 years. The trial will evaluate potential preventive treatments for certain clinical conditions which are important causes of morbidity and mortality in postmenopausal women.

The trial will have three main components and four active treatments. The treatments will be tested in a partial factorial design (*Figure 1*). Such a design allows the total number of participants to be considerably less than would be required for separate experiments for each of the three CT components. The first component will test separately the efficacy of ERT vs. placebo among hysterectomized women, and of PERT vs. placebo among women with a uterus*, on CHD; the second will test the efficacy of low-fat dietary pattern vs. usual dietary pattern on breast and colorectal cancers (two arms); and the third will test the efficacy of CaD supplementation vs. placebo on hip fractures (two arms). In regard to safety, clinical outcomes of interest include breast cancer and endometrial cancer (HRT) and renal calculi (CaD supplementation).

Sample size calculations indicate that for the HRT component, 27,500 women, and for the DM component 48,000 women, treated for an average of nine years would provide adequate power for the primary outcomes of interest. Assuming some overlap between the HRT and DM components, it is anticipated that a total of 64,500 women would enter the CT. It is assumed that about 45,000 (70%) of these women will be willing to subsequently enter the CaD component (see *Section 1-A3, Protocol Appendix 3* for statistical power calculations). Post-trial mortality and breast and endometrial cancer incidence surveillance for a further five years is envisaged, so that total follow-up will be for an average of 14 years. The longer follow-up will protect against the possibility of missing adverse effects, such as breast cancer in relation to HRT, which may not have had sufficient time to manifest clinically during the nine year average follow-up period.

Women will be recruited on the basis of their eligibility and willingness to participate in either the HRT or the DM components, or both. It is anticipated that about 40% of women who are enrolled in the HRT component will also be enrolled in the DM component. This 40% rate is the product of 0.60, the fraction of HRT women who are expected to meet DM-specific eligibility criteria (*Protocol Section 4.4*), and 0.67, the fraction of HRT women who are assumed to be willing to be randomized into the DM component. A smaller proportion (23%) of women in the DM component are expected to also be eligible and willing to enter the HRT component. The distribution of women in the dietary component will be 40:60 active treatment:control, and in the HRT 9:11:20 ERT:PERT:placebo as is elaborated in the following paragraph. The unequal distributions are intended to decrease study costs while maintaining statistical power. The randomization in the CaD component, which will take place at a participant's first annual visit, will be 50:50 active treatment:placebo. The total trial cohort size is projected to be 64,500.

Women who are post-hysterectomy will be randomized in the HRT between placebo and ERT in the ratio of 1:1. PERT will not be an option for such women, as the role of the progestin is primarily to protect the uterus. Women with an intact uterus will be randomized to placebo or PERT in the ratio of 1:1. The fraction of women with a hysterectomy at baseline will be restricted to be approximately 45%. This distribution was chosen in order to achieve adequate power for ERT vs. placebo and PERT vs. placebo in respect to CHD. *Figure 1* shows the projected number of women in each cell of the CT defined by the HRT and DM randomizations.

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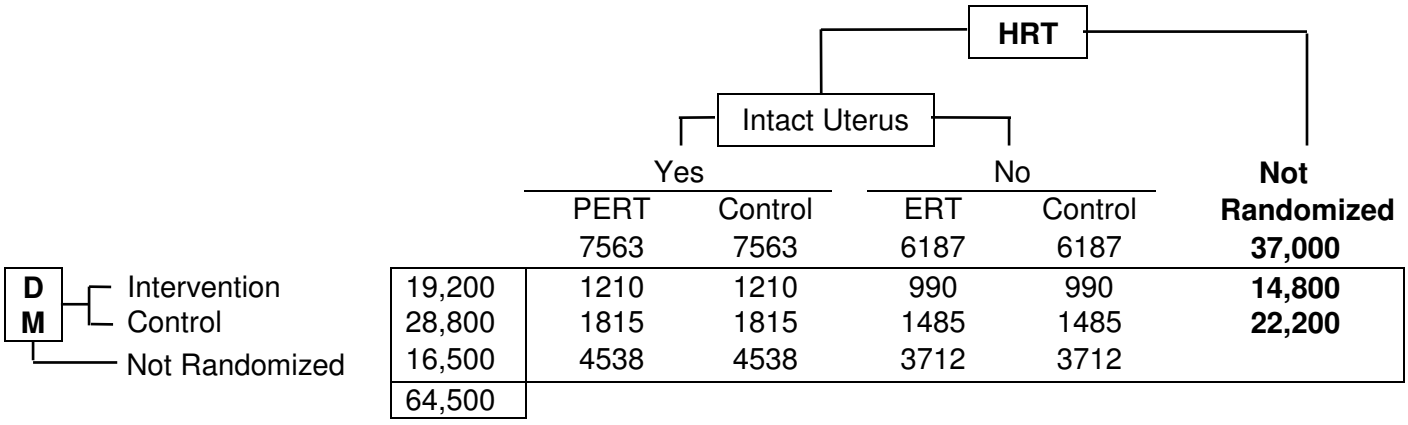
* In the original design, women with a uterus could also be randomized to ERT with annual endometrial monitoring. The PEPI experience (PEPI, 1995) clearly indicated that the unopposed estrogen arm was infeasible because of an unexpectedly high incidence of endometrial hyperplasia. Randomization to ERT among women with a uterus was therefore stopped on December 16, 1994. The 331 women who had previously been randomized to ERT were changed to PERT. They will be followed in the PERT arm for routine analyses. Separate analyses of women randomized before December 16, 1994 or additional stratification to account for this change will be used in key analyses.

Figure 1

WHI CT Partial Factorial Design

Projected number of women entering the various trial components* :



The frequency of women by age group will have the following targets, with acceptable age ranges given in parentheses: 10% (0-15%) for ages 50-54; 20% (15-25%) for ages 55-59; 45% (40-50%) for ages 60-69; and 25% (20-30%) for ages 70-79. These frequencies were motivated by a desire to retain the entire age range 50-79, while paying suitable attention to overall risk versus benefit projections. Power calculations (*Section 1-A3, Protocol Appendix 3*) have been based on the above age targets. To achieve the designated power, accrual of women into both the HRT and the DM components will be restricted to the given age-specific ranges in each clinic.

Women aged 50-79 who have been screened and found not to be eligible for the trial, or who after screening are not willing to participate in the trial, will be invited to participate in the OS component. Supplemental recruitment to the OS may be required in some CCs. Much of the same baseline information as for women in the trial will be collected, and mortality and morbidity surveillance will be maintained for an average of nine years. A supplemental epidemiologic questionnaire will also be administered to OS women. In addition, women will be invited to attend a second visit three years after baseline, in order to allow examination of the effects of changes in characteristics on disease outcomes. It is anticipated that 100,000 women will be recruited into the OS.

4.2 Choice of Treatments

4.2.1 Hormones

As previously mentioned, women participating in the HRT component will be randomized based on the presence or absence of a uterus.

1. Women with a uterus will be randomized to one of two arms:
    - Conjugated equine estrogen (CEE) 0.625 mg per day plus medroxyprogesterone* (MPA) 2.5 mg per day continuously (PERT)

* In each cell, approximately 70% of women are projected to be eligible and willing to be randomized to receive calcium and vitamin D supplementation or placebo (1:1 allocation).

- Placebo estrogen plus placebo progestin

2. Women without a uterus will be randomized to one of two arms:
   - Conjugated equine estrogen (CEE) 0.625 mg per day (ERT)
   - Placebo estrogen

The drug manufacturer will provide the single and combined hormones in single tablets, so that all participants will take only one tablet per day, regardless of the arm to which they are randomized. The drugs will be distributed in 6 - to 12 - month supplies in bottles that women will return for tablet measuring and replacement.

Drug dosages and regimes were chosen to minimize side effects and adverse effects, and provide ease of administration while maintaining clinical effectiveness.

The selected hormones and the rationale for these choices are as follows:

1. Conjugated equine estrogens (CEE) at a dose of 0.625 mg/day:

   This lower dose and type of estrogen is associated with favorable changes in blood lipids, bone loss, and coronary risk, and may be less likely to increase rates of breast cancer and endometrial cancer. Furthermore, conjugated equine estrogens are the most commonly prescribed estrogen preparation in the United States.

2. Medroxyprogesterone (MPA) at a dose of 2.5 mg/day, continuous:

   This agent was chosen from among the progestins because at this continuous low dose it appears to cause less reversal of the beneficial effects of estrogen on lipids than the higher dose cyclic MPA, or the 19-nortestosterone derivatives. It is the most widely prescribed progestin in the United States and is also believed to be as protective of the endometrium as higher dose cyclic MPA. Many women are reluctant to have regular menstrual periods that occur in 85% of women on cyclical regimes. In addition, there will be the benefits of ease of administration for the participants, as well as facilitation of blinding, timing of clinic visits, and drug packaging.

4.2.2 Dietary Modification Component Goals

The nutritional goals for the intervention group are to reduce the intake of total dietary fat to 20% of corresponding daily calories, reduce the intake of saturated fats to less than 7% of calories, and to increase servings of vegetables and fruits to five or more daily and servings of grain products to six or more daily. Each participant's fat intake goals will be expressed in grams of fat per day. The fat gram goal will be calculated using an algorithm based on height and a fat gram goal of 15% energy from fat, calculated using expected caloric intake after one year of intervention (*Table 1*).

Table 1
Fat Gram Goals for DM Component Based on Height

Height (cm)	Fat Gram Goal
150	23
152	23
154	24
156	24
158	24
160	24
162	24
164	25
166	25
168	25
170	25
172	26
174	26
176	26
178	26
180	27

Dietary Control Group

Women in the DM control group will not be offered a nutrition intervention program since the general strategy to be adopted for this group will be minimum interference with customary diets while collecting nutritional data considered appropriate for comparison with the nutrition intervention group. Participants in the control group will be provided a standard packet of health promotion materials including information on basic nutrition principles for maintaining nutritionally adequate diets, and a copy of the USDA/DHHS Dietary Guidelines for Americans.

Dietary Intervention Procedures

Women randomized to the DM intervention arm will be assigned to a permanent group of 8-15 members led by a designated nutritionist. Each such woman will attend her first group meeting within 12 weeks of randomization. The first meeting for each group will be within four weeks of its formation. The group will meet weekly for six weeks, bi-weekly for six weeks, and monthly for nine months. Each woman will have an individual counseling session with her Group Nutritionist between weeks 12 and 16 from the beginning of intervention sessions. The importance of attendance at scheduled sessions will be emphasized. If a participant misses a group session she will be strongly urged to complete make-up activities. All dietary intervention women will receive a packet of health promotion materials similar to that of control participants but without dietary information.

Self-monitoring tools (Food Diary with Fat Counter and Fat Scan) will be used as educational and monitoring aids during the first year of intervention and a shorter alternative tool will be used during maintenance. For early monitoring of adherence with dietary fat goals, the fat scores calculated from the Food Diary and Fat Scan collected from group sessions 4, 8, 12 and 16, and from the individual counseling visit, will be entered into the CT database. Consumption of fruits, vegetables, and grain products will be self-monitored at these same time points in the first year. The intervention integrates knowledge and skills in both nutritional and behavioral sciences. It uses a small group format and a self-reliant, self-directed approach. Self-monitoring and self-correction have been shown in extensive feasibility testing to produce dietary changes. There is individual flexibility about the exact changes in dietary composition and the rate at which they are made. The

information and skills presented during the group sessions build upon the content of previous sessions and provide opportunities for necessary practice, feedback, and reinforcement. All the knowledge and skills required to bring about the dietary change goals are covered during the first year of intervention. Throughout the first year, the intervention will be delivered according to a standardized protocol in all clinics.

Weight reduction and reduction in total calories are not stated goals of the nutrition intervention. Neither body weight nor dietary caloric consumption will be controlled, but reductions in both are expected to accompany successful intervention. Maintenance of dietary change will begin in the second year and will involve about four meetings each year. The meetings will provide opportunities to update nutritional information, and review and practice skills that aid in the maintenance of dietary change. Intervention groups seeking added social support will be encouraged to meet more frequently under the guidance of "peer group leaders." "Peer-led help groups" will be discussed during the last six months of year one. In addition to the planned quarterly meetings, there will be two large group social functions yearly. The emphasis will be to promote social support among group members and between intervention groups.

After the first year, 2 - 4 newsletters will be sent to all CCs for use with their intervention participants. Some variation will be allowed during the maintenance phase in the delivery of the intervention. *Vol. 2 - Procedures* will define the range of variation allowed.

Section 1-A6, Protocol Appendix 6 provides some further detail on the intervention program and dietary assessment methods.

4.2.3 Dose and Preparation of CaD

CT women will be asked at their first annual visit if they are interested in joining the CaD component. Willing and eligible women will be randomized in the ratio of 1:1 to one of two arms:

1. Calcium carbonate containing 1000 mg elemental calcium per day plus vitamin D_3, with meals. Women will be given a choice of taking chewable or swallowable pills.

    - Each will contain 1000 mg elemental calcium per day plus vitamin D_3, up to a maximum of 400 International Units (IU) per day. This will be dispensed as two tablets.

2. Placebo calcium and placebo vitamin D, with a meal, also dispensed as two tablets. Both a chewable and swallowable placebo will be available to correspond to the chewable and swallowable active tablets.

The manufacturer will provide the active and placebo supplements in single chewable tablets. The supplements will be distributed in 6 - 12 month supplies in bottles. Women will be asked to return all bottles for tablet measuring and replacement.

The dose and preparations of calcium were chosen for ease of administration, satisfactory blood absorption, and low frequency of hypercalciuria. This type of supplement is available over-the-counter and has been widely used in the United States for many years. Women will be encouraged, but not required, to take the two pills at different mealtimes, each day. The dose of 1000 mg per day aims to yield an average total calcium intake in excess of 1500 mg per day of elemental calcium in the active treatment group.

The dose of 250 to 400 IU of vitamin D_3 is large enough to ensure adequacy (RDA is 200 IU daily), without risking toxicity. This is a typical dose for supplementation in multivitamin tablets, and has been found to raise 25-hydroxy vitamin D_3 concentrations to acceptable levels (Ohmdahl et al., 1982; Webb et al., 1990), and to slow bone loss (Dawson-Hughes et al., 1991). This dose can be given safely without risking hypercalciuria.

4.2.4 Exercise Advice

All randomized women will receive advice and a pamphlet encouraging them to follow a program of moderate exercise (e.g., including walking briskly for half an hour per day).

4.3 Outcomes of Interest

4.3.1 Major Clinical Outcomes

Clinical outcomes are divided into primary outcomes for the CT (with sufficient power for detection in a pertinent CT component), subsidiary outcomes of interest (but not necessarily with adequate power), and composite outcomes (combinations of primary and subsidiary outcomes). The primary outcome for the HRT component is fatal and non-fatal CHD; for the DM component, breast cancer and colorectal cancer separately; and for the CaD component, hip fractures.

Three general classifications of morbidity define major clinical outcomes for the CT and OS: CVD, cancer, and fractures. Mortality will also be an important clinical outcome, and will include all-cause and cause-specific mortalities (e.g., CHD, other CVD, and cancer). Clinical outcomes in the CT will be initially identified by semi-annual self-administered questionnaires, and in the OS by annual self-administered mailed questionnaires, with telephone follow-up as needed. After initial identification, clinical outcomes in the OS will be ascertained and classified in the same way as in the CT with some minor differences in the extent to which outcomes are adjudicated. *Volume 8 - Outcomes* provides further detail on the outcome ascertainment and classification plan. Each outcome category listed below will be ascertained separately.

1. Primary outcomes:
   - A. Coronary heart disease:
     - acute myocardial infarction requiring overnight hospitalization
     - coronary death
   - B. Cancer
     - Breast
     - Colorectal
   - C. Hip fracture
2. Subsidiary outcomes:
   - A. Cardiovascular disease
     - Acute (including aborted) myocardial infarction (fatal or non-fatal requiring overnight hospitalization)
     - Coronary death (sudden and non-sudden)
     - Stroke (fatal and non-fatal requiring overnight hospitalization)
     - Congestive heart failure (requiring overnight hospitalization)
     - Angina pectoris (requiring overnight hospitalization)
     - Peripheral vascular disease (requiring overnight hospitalization)
     - Coronary revascularization
   - B. Other Cancers
     - Colon
     - Rectum
     - Endometrium

- Ovary

C. All other fractures

D. Venous thromboembolic disease requiring overnight hospitalization

- Pulmonary embolism
- Deep venous thrombosis

E. Diabetes mellitus requiring therapy

F. Other age-related outcomes

- Inflammatory arthritis
- Glaucoma
- Urinary incontinence
- Physical function status
- Cognitive function and dementia

3. Composite outcomes:

A. Cardiovascular disease

- Major: CHD, stroke, or congestive heart failure requiring overnight hospitalization, peripheral vascular disease with amputation
- Any: major CVD plus congestive heart failure, other peripheral vascular disease, coronary revascularization, or angina requiring overnight hospitalization

B. Cancer

- Diet-related: breast, colorectal, endometrial, ovarian
- Hormone therapy-related: breast, endometrial
- Total cancer (exclusive of non-melanoma skin cancer)

C. Any fracture

D. Any hospitalization (except certain elective procedures listed in WHI Manuals *Vol. 8: Outcomes Procedures*)

E. Total mortality

F. Cause-specific mortality

- Atherosclerotic cardiac disease
- Cerebrovascular disease
- Other CVD
- Cancer
  - Diet-related: breast, colorectal, endometrial, ovarian
  - Hormone therapy-related: breast, endometrial
  - All cancer

G. Violent/Accidental/Suicide

H. Other deaths

All clinical outcomes will be monitored in all participants in the CT and the OS. Selected outcomes are related to specific program components as shown in *Table 2*.

Table 2
Outcomes for WHI CT and OS

"1°" indicates primary outcomes; "2°" subsidiary and composite outcomes; "x" ascertained

Outcome	HRT	DM	CaD	OS
CARDIOVASCULAR:				
Coronary heart disease	1°	2°	x	x
Stroke	2°	2°	x	x
Congestive heart failure	2°	2°	x	x
Angina	2°	2°	x	x
Peripheral vascular disease	2°	2°	x	x
Coronary revascularization	2°	2°	x	x
Total cardiovascular	2°	2°	x	x
CANCER:				
Breast cancer	2°	1°	2°	x
Endometrial cancer	2°	2°	x	x
Colorectal cancer	x	1°	2°	x
Ovarian cancer	2°	2°	x	x
Total cancers	2°	2°	2°	x
FRACTURES:				
Hip	2°	x	1°	x
Other fractures	2°	x	2°	x
Total fractures	2°	x	2°	x
Venous thromboembolic disease				
Pulmonary embolism	2°	x	x	x
Deep vein thrombosis	2°	x	x	x
Diabetes mellitus requiring therapy	x	2°	x	x
Death from any cause	2°	2°	2°	x

4.3.2 Intermediate Outcomes

In addition to clinical outcomes of interest to the WHI, other findings determined by laboratory, radiologic, pathologic or physical examination will serve as intermediate outcomes. A detailed schedule for measurement of intermediate outcomes during the follow-up period is included in *Volume 8 - Outcomes.* Some of these outcomes will be ascertained in all participants and others in defined subsamples of participants.

Most intermediate outcomes will be measured at baseline and one year of follow-up in CT participants to assess short-term effects of treatment. These outcomes will then be measured in a subsample at three, six, and nine years after randomization. For example, prospective laboratory analyses of a subsample of CT participant blood specimens for fasting glucose, lipids/lipoprotein, fibrinogen, factor VII and antioxidants are planned. Intermediate outcomes related to safety are assessed on a schedule determined to be appropriate for the intervention under test. Intermediate outcomes will be measured at baseline and three years of follow-up in OS participants. Some laboratory outcomes will only be measured in a subsample.

4.4 Study Population

The eligibility and exclusion criteria are as broad as possible in order to increase the generalizability of the results to the population of postmenopausal women. The trial will combine primary and secondary prevention. Thus, women with prevalent CVD, and women with a past history of fractures, will be included (with some exclusions noted below). The study population can be drawn from a convenient population (e.g., a clinic-

based sample), the general population (population-based sample), or a combination of both. In view of cost considerations and the near-impossible goal of obtaining a truly representative population sample for a clinical trial, it is anticipated that most of the participants in the CT/OS will be drawn from convenient populations. Though this does limit the generalizability of estimates of disease and risk factor prevalence, it is expected to have a minimal impact on the generalizability of treatment effects or relative risk estimates.

To maintain power, some restrictions will be made on accrual for defined subgroups. In all components, as mentioned above, the target fractions for randomized women will be 10%, 20%, 45%, and 25% for ages 50-54, 55-59, 60-69, and 70-79, respectively. In the HRT, hysterectomized women will represent a target 45% of the accrued population. These distributions will be monitored within each CC and enrollment may be temporarily closed to appropriate subgroups to achieve these goals. Social/ethnic minority women will be represented in the overall sample with a target of at least the proportion that they are found in the general population of women age 50-79 (17% according to the 1990 census) with a specific target of 20% minority women in the CT/OS. Efforts will be made to ensure adequate representation of minority women and women of lower socioeconomic status, primarily by including CCs having access to large numbers of women in such population subgroups.

In the planning stage it was anticipated that 45 CCs would participate. Recruitment goals for each CC are therefore defined as 1/45 of the study-wide goal for study component and age. To fulfill the additional recruitment needs created by the funding of only 40 CCs, existing CCs will be asked to apply for funding to extend their recruitment beyond original goals.

Inclusion Criteria for All Components

* 1. Postmenopausal female volunteers of all races and ethnicity, with or without a uterus or ovaries (see *Vol. 2 - Procedures* for detailed procedures for establishing menopausal status).

* 2. Ages 50-79 years, inclusive, at first screening contact.

* 3. Likely to be residing in study area for at least three years after randomization or enrollment.

4. Providing written informed consent.

Exclusion Criteria

A. Exclusion Criteria for All Components

1. Competing Risk

* a. Any medical condition associated with predicted survival of less than three years in the judgment of a Clinic physician (e.g., class IV congestive heart failure, obstructive lung disease requiring long-term ventilation or supplemental oxygen in the past, severe chronic liver disease with jaundice or ascites, kidney failure requiring dialysis, sickle cell anemia)

2. Adherence or Retention Reasons

* a. Alcoholism

* b. Other drug dependency

c. Mental illness, including severe depression

d. Dementia

* e. Active participant in any other interventional trial where participants are individually randomized to an intervention or control group

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**Note*: An asterisk (*) in the above listing implies that a woman who is temporarily excluded may be re-evaluated for eligibility as appropriate to the excluding condition. If more than six months have elapsed since the woman's SV1, however, most baseline and screening activities must be repeated.

B. Additional Exclusion Criteria for All CT Components

1. Competing Risk

   a. Invasive cancer of any type in the past 10 years

   b. Breast cancer at any time (in situ or invasive)

   * c. Baseline mammogram or clinical breast examination findings suspicious of breast cancer (see *Vol. 2 - Procedures* for detailed criteria)

   * d. Acute myocardial infarction in past six months

   * e. Stroke or transient ischemic attack (TIA) in the past six months

   f. Known chronic active hepatitis or severe cirrhosis

2. Safety Reasons

   ** a. Severely underweight (recommended limit** of BMI < 18 kg/m^2 or unintentional loss of 15 or more pounds in previous six months)

   * b. Hematocrit < 32%

   * c. Platelets < 75,000 cells/ml

   * d. Severe hypertension (systolic BP > 200 mmHg or diastolic BP > 105 mmHg)

   e. Current use of oral corticosteroids

3. Adherence or Retention Reasons

   a. Unwilling to participate in baseline or follow-up examination components such as mammograms, clinical breast exams, phlebotomy, electrocardiograms, questionnaires and forms; or unable to complete baseline study requirements

4. (Bone Densitometry Clinics) Femoral neck bone mineral density of more than 3.0 standard deviations below the age specific mean.

C. Additional Exclusion Criteria for Hormone Replacement Component

1. Safety Reasons

   a. Endometrial cancer of any stage at any time

   b. Endometrial hyperplasia at baseline (no recycling)

   c. Malignant melanoma of any stage at any time

   d. History of pulmonary embolism or deep vein thrombosis

   e. Previous osteoporosis-related fracture being treated with HRT

   f. History of bleeding disorder serious enough to require transfusion

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*_Note_: An asterisk (*) in the above listing implies that a woman who is temporarily excluded may be re-evaluated for eligibility as appropriate to the excluding condition. If more than six months have elapsed since the woman's SV1, however, most baseline and screening activities must be repeated.

** Recommended limits are given for aspects where Clinician judgment may be used to evaluate eligibility on a case-by-case basis.

g. Lipemic serum leading to diagnosis of hypertriglyceridemia (>500 mg/dl) on baseline blood draw

* h. Currently on anticoagulants

i. Currently on tamoxifen or other selective estrogen receptor modulators

* j. Abnormalities in baseline PAP smear, pelvic exam or pelvic ultrasound (if performed)

2. Adherence or Retention Reasons

a. Severe menopausal symptoms that would make placebo therapy intolerable to the participant

* b. Inadequate adherence with placebo run-in (less than 80% of daily pills taken) (only one repeat run-in period is allowed)

c. Unable or unwilling to discontinue use of HRT (women must discontinue current replacement hormone therapy for at least three months prior to baseline measures for HRT enrollment)

d. Unable or unwilling to discontinue use of oral or injectable testosterone (must discontinue current testosterone use for at least three months prior to baseline measures for HRT enrollment)

e. Unwilling to have baseline or follow-up endometrial aspirations (women with a uterus)

D. Additional Exclusion Criteria for Dietary Modification Component

1. Adherence or Retention Reasons

* a. Special dietary requirements incompatible with the intervention diet (such as celiac sprue, other malabsorption syndromes). Women will be eligible if they are following a diabetic diet or a low salt diet.

b. Colorectal cancer at any time

c. Unable to complete Four-Day Food Record adequately

* d. FFQ percent of calories from fat below a cutpoint chosen to exclude about 40% of screened women (may repeat assessment after 1 month). FFQ energy intakes of < 600 kcal or > 5000 kcal at screening.

* e. Number of main meals prepared out of home ≥ 10 per week

f. Type I (insulin-requiring, ketosis-prone) diabetes mellitus

g. Gastrointestinal conditions that contraindicate a high fiber diet

h. Bilateral prophylactic mastectomy

E. Additional Exclusion Criteria for CaD Component

1. Competing Risk: The following "all components" and "all CT components" exclusion criteria will be reassessed just prior to randomization into the CaD component.

a. Any medical condition associated with predicted survival of less than three years as described above (A.1.)

2. Safety Reasons

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*_Note_: An asterisk (*) in the above listing implies that a woman who is temporarily excluded may be re-evaluated for eligibility as appropriate to the excluding condition. If more than six months have elapsed since the woman's SV1, however, most baseline and screening activities must be repeated.

a. History of renal calculi

b. History of hypercalcemia

c. Current use of oral corticosteroids

d. Continuing use of > 600 IU Vitamin D

e. Current use of calcitriol

3. Adherence or Retention Reasons

a. Dementia

4.5 Sample Size and Duration

To have sufficient power to test the primary hypotheses, it is estimated that the CT will need to randomize 27,500 women into the HRT component to be followed for an average of nine years; 48,000 women into the DM component to be followed for an average of nine years. An estimated 45,000 women will be randomized into the CaD component to be followed for an average of eight years. For details of the sample size calculations, see *Section 1-A3, Protocol Appendix 3*. The total sample size required to achieve the above sample size targets will depend on the proportion of women willing to participate in more than one CT component. Only women who are potentially eligible and interested in the HRT or DM components will be invited for an SV1. The total sample size of 64,500 shown in *Figure 1* is based on the assumption that 40% of the women who choose the HRT component will also be eligible and willing to be randomized to the DM. It is further assumed that about 45,000 of these 64,500 women (70%) will be eligible and willing to participate in the CaD component, for which randomization will typically take place at the participant's first annual visit (*Figure 1*). It is envisaged that CT women will be followed for mortality and for breast and endometrial cancer incidence for an additional five years beyond the end of the nine year average follow-up period mentioned above. This will allow more precise safety evaluations and total mortality comparisons for the CT.

Assuming that approximately one-third of women who attend SV1 will be enrolled into the CT, we anticipate that approximately 100,000 women will be entered in the OS. This sample size will provide adequate power to obtain precise estimates of the strength of risk factors, since substantial numbers of clinical events can be expected to occur (*Table 3*, see also *Section 1-A3, Protocol Appendix 3*). It may be necessary to recruit some women directly to the OS in order to meet this 100,000 recruitment goal.

Table 3
OS - Estimates of Cumulative Number of Events For 100,000 Women Age 50-79 at Screening Visit

Average Years of Follow-Up	Total Deaths	CHD	CVD	Breast Cancer	Colorectal Cancer	Selected *Fractures
3	5,000	1,900	4,000	1,000	500	3,300
6	11,100	4,200	8,500	2,000	1,100	7,000
9	18,200	6,700	13,800	3,100	1,900	11,200

* Indicates hip, pelvis, vertebrae, distal radius and proximal humerus fractures

4.6 Informed Consent

The participant's full understanding of the pertinent study components is important for ethical reasons and for adherence with study protocol. Verbal consent will be obtained from each woman who is contacted by phone and asked screening questions. At the beginning of SV1, each woman will be given general information about the CT components and the OS, and she will be given the opportunity to view a video describing the study. A general limited informed consent will be obtained at this time for the initial screening activities, including processing of questionnaire data, drawing blood, and obtaining medical records. Material written in large print in 6th grade level English or Spanish, that describes the study in general terms will be given. Toward the end of SV1, if the woman is deemed eligible, the components for which she is eligible and interested will be described in detail, and written material describing each pertinent CT component will be provided. After she has had the opportunity to read and discuss this information with the study personnel, she will be given a copy

of the informed consent form to take home and review. At the beginning of Screening Visit 2 (SV2), each woman continuing to be CT eligible and interested will be given an opportunity to ask additional questions. She will then be asked to make a decision regarding her participation in the HRT and DM components, and if such decision is positive, she will be asked to sign a consent form specific to the components she plans to enter.

Written material on the CaD component will be provided to CT women prior to their first annual visit, along with a copy of a corresponding consent form. At the beginning of the first annual visit, each CT woman will have the opportunity to discuss the CaD component with study personnel, and to ask related questions. Eligible women will then be asked to make a decision concerning participation in the CaD component and if such a decision is positive, will be asked to sign an informed consent form specific to this CT component.

Women who express initial interest but turn out to be ineligible or unwilling to enter the CT at any point in the screening process will be invited to participate in the OS if they meet OS eligibility criteria. In order to meet OS recruitment goals some women may be invited to be screened for the OS regardless of initial interest in the CT. Interested women will be asked to sign an OS informed consent form. Model informed consent forms are listed in *Section 2 - Consent Forms*. Clinical Centers are allowed to modify these consent forms only for language and clarification. All consent forms must be submitted to the Project Office for approval.

4.7 Randomization Assignment Blinding

4.7.1 Hormone Replacement Therapy*

All clinic personnel and participants will be blinded to individual treatment assignments. All efforts will be made to prevent unblinding of participants for the duration of the trial. However, in some instances of unexpected or abnormal uterine bleeding, or of serious adverse experiences, it may be necessary for a clinic consulting gynecologist or a private physician to be unblinded to ensure maximal patient safety.

Some amount of spotting is expected during the first six months, particularly in the PERT arm of the HRT component, but this may resolve. Each site will identify an individual(s) ("designated clinic contact(s)") who will be trained to give uniform and consistent advice to participants calling to report bleeding during the first six months of therapy. During the first year, a designated clinic contact will be responsible for reviewing the bleeding calendar as well as interacting with participants to follow-up on episodes of bleeding. Because of the likely association between bleeding (and other) symptoms and treatment assignment, this individual should not be involved in ascertainment and adjudication of outcomes. Should a designated clinic contact need to consult with other clinic staff who are following the participants, she/he should make every effort to describe the woman's symptoms without identifying the person.

Depending on the clinical findings, unblinding will be considered under circumstances involving either participant safety or management of side effects. Such conditions are discussed in *Protocol Section 5.5* and *Vol. 2 - Procedures*. Should unblinding become necessary, the Clinic Unblinding Officer will exercise a database algorithm, detailed in *Vol. 2 - Procedures*, that confirms that unblinding criteria have been met, and that records the unblinding activity in the database. The unblinded information will be restricted to the Unblinding Officer and the clinic consulting gynecologist. The Unblinding Officer will be a CC staff person without other participant interaction responsibilities (e.g., a data coordinator or support person). As long as the unblinded information is limited to these individuals and these persons are not involved in outcome adjudication, the potential for biasing study outcomes is minimal. Bias can further be minimized by maintaining participant blinding, even when unblinding of the consulting gynecologist becomes necessary.

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* On July 9, 2002, the intervention for the PERT arm was stopped. After an average follow-up of 5.2 years, the DSMB concluded that the risks of taking active estrogen plus progestin outweighed the benefits (Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: Principal results from the Women's Health Initiative randomized controlled trial. JAMA. 2002;288:321-33). The Estrogen-Alone trial continues without change.

Serious complications such as those requiring surgery may, of course, necessitate unblinding a small number of participants. The same unblinding mechanism will be used in the event of study medication overdose by a participant or other person (see *Vol. 2 - Procedures*).

4.7.2 Dietary Modification

This trial will, of necessity, be unblinded. However, personnel investigating and classifying laboratory determinations and other intermediate data, as well as clinical outcomes of interest, will be blinded to the participants' randomization group.

4.7.3 Calcium/Vitamin D

All efforts will be made to maintain double-blinding throughout. The same mechanism alluded to above will be used to unblind in the event of overdose.

4.7.4 Coordinating Center Blinding

Access to individual participant's treatment assignment(s) by Clinical Coordinating Center (CCC) personnel will be strictly limited and based on need-to-know criteria. Functions requiring treatment assignment information in the CCC include quality assurance and reporting. For quality assurance of CC unblinding, it will be necessary for CCC medical personnel to review unblinding occurrences at CCs to determine the appropriateness of unblinding and the adequacy of follow-up. For those cases, CCC medical personnel involved in these quality assurance procedures will be prohibited from any subsequent outcome ascertainment or adjudication procedures for those participants whose treatment assignments were revealed to them.

For reporting purposes, access to individual treatment assignment will be limited to analytic and programming staff. The Statistics and Data Management Units of the CCC will produce all routine reports on primary and intermediate outcomes, safety issues, and laboratory determinations by treatment arm for examination by the Data and Safety Monitoring Board (DSMB). Distribution of these reports will be limited to the DSMB members, appropriate NIH staff, the CCC PI, and necessary Statistics and Data Management staff. To reduce further the risk of unauthorized release of information, the following steps will be taken: in preparing the analyses and reports, all CCC staff involved will be reminded of the nature of the data and reports; working versions of all output will be shredded prior to disposal; and CCC personnel not involved in producing reports will not be given access to these documents. After each review the reports will be collected and stored centrally in a secure location. For study management purposes, summary reports accumulated across treatment arms will be presented to display overall study progress.

5. Study Plan

5.1 General

The feasibility of funding and completing a study of this size and duration depends on cost-effective methods of recruitment, intervention, and follow-up. Clinic visits will be kept to a minimum frequency and duration. Only data that are essential to answering the study hypotheses and to the safety of participants will be gathered routinely. Considerable effort will be made to ensure complete and accurate ascertainment of clinical outcomes. However, for many intermediate questionnaire data, physical measurements, and biochemical variables, strategies to contain data proliferation and cost will be developed and implemented, including the freezing of samples to allow retrospective measurement among cases and controls, obtaining data on subsamples of the study population only, and obtaining data at extended intervals rather than annually.

For example, the effects of a particular treatment on an intermediate variable over time will be assessed by making observations at extended intervals or in an appropriate subsample. It is envisaged that repeated measurements on a random sample of about 5% of the total CT population (with oversampling of racial/ethnic minorities and of HRT vs. DM women) will be sufficient to provide information on trends over time for the intermediate outcomes.

Secondly, to allow the relationship of change in intermediate variables to clinical outcomes to be evaluated, various observations will be made in all CT participants at entry and after one year, and of all OS women after three years. However, analyses of most samples will be confined to subsequent cases and appropriate controls.

5.2 Enrollment

5.2.1 General Screening

The major activities and flow for screening are presented in *Figure 2* and *Figure 3*. Recruitment activities will be targeted toward women eligible for and interested in the CT, though some recruitment of women directly into the OS may ultimately be necessary to meet OS recruitment goals. An eligibility screen is planned prior to the first clinic visit in order to minimize clinic burden.

The activities listed in *Figure 2* and *Figure 3* represent a model screening scenario. Departures from this scenario may be exercised at clinic options as is elaborated in subsequent subsections. In particular, activities may be moved forward in the screening process provided that pertinent participant consent has been obtained. CT women who do not complete all required screening activities within six months of their SV1 will be required to provide updated baseline information prior to randomization. Women may prove to be ineligible or unwilling for CT enrollment at various points in the screening process. Such women will be offered the opportunity to participate in the OS and will be asked to complete certain OS baseline activities prior to leaving the clinic. In the event that a fasting blood sample has not been obtained, such women will need to return to the clinic for a fasting blood draw prior to OS enrollment.

A three-year recruitment period is anticipated for each CC. *Section 1-A1, Protocol Appendix 1* provides a more detailed list of the measures to be collected at each clinic visit.

5.2.2 Pre-Screening

Multiple recruitment strategies may be needed at each CC. To meet the high recruitment goals of the program, it is recommended that mass mailings be used to produce a steady flow of interested, potential participants for the initial screening. Addresses for these mass mailings can be obtained from such sources as motor vehicle registration lists, drivers' license lists, HMOs, HCFA, health insurance companies, and commercial mailing lists. These can be supplemented with the following:

1. Media efforts: Use of newspapers, radio, TV, newsletters

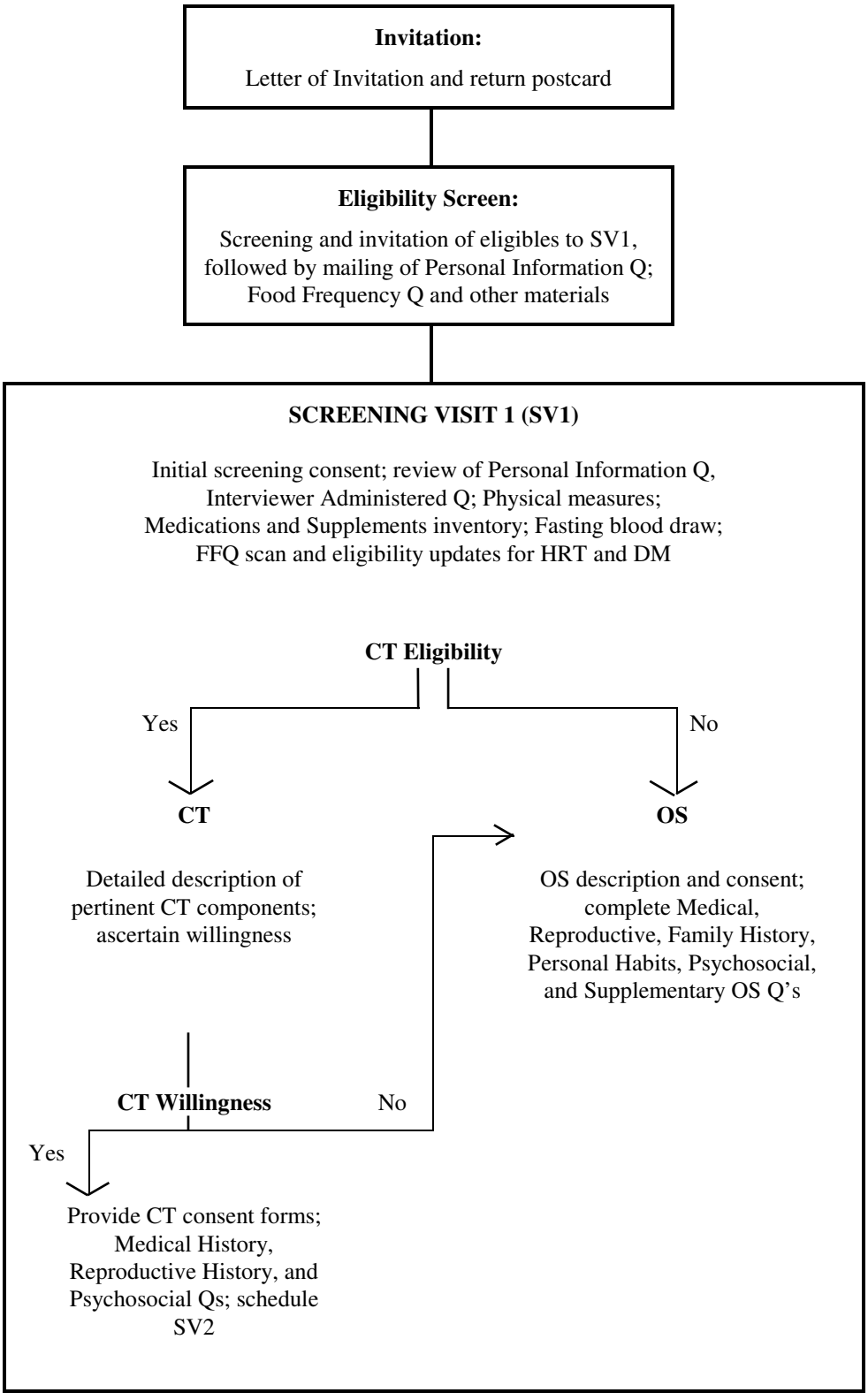
2. Mass screening of certain high yielding communities (e.g., senior housing facilities), special social groups (churches), special occupational groups
3. Blood banks
4. Laboratory lists
5. Medical referrals
6. Mammography screening centers

The model initial contact will involve a letter and/or brochure providing basic information on the WHI and a postage-paid postcard to be returned indicating interest in participation. Age-eligible women indicating an interest will be contacted by phone by trained interviewers to identify women ineligible for the CT. Women interested in HRT who are currently taking hormones will be asked to discontinue hormone use for three months prior to proceeding with the screening visits. These women will be encouraged to discuss this with their personal physician and CCs will provide informational materials to these women and physicians as needed. Women ineligible for both the HRT and DM components will be thanked for their time and interest, and contact information on such women will be retained for possible later contact in relation to the OS. Those continuing eligible for the CT will be scheduled for a First Clinic Visit (SV1), and will be instructed that a packet of materials will be mailed to them for their attention before SV1. The packet will include a cover letter, directions and information for travel to the clinic, a logo bag for all of their current prescriptions and regularly used over-the-counter medications and vitamin supplements, a personal information form and an FFQ. Women should be scheduled for SV1 as soon as possible after their initial interview. Women given appointments before noon will be asked not to eat or drink anything except water for 12 hours before their appointment and if possible to refrain from smoking and vigorous exercise for 12 hours prior to the appointment in preparation for a blood draw. Clinical Centers should schedule as many SV1s as possible in the morning so that the blood sample can be obtained during this first visit.

There are a number of clinic options that can be exercised between the initial contact and SV1. For example, clinics wanting to maximize the HRT/DM overlap may choose to selectively invite women continuing eligible for both the HRT and DM to SV1. Clinics wanting to minimize SV1 visits that do not lead to CT enrollment could request that the FFQ be returned by mail and scanned in order to avoid visits for women who are eligible for neither CT component, or for the HRT only. Clinics concerned about the ability of women to complete an FFQ and Personal Information Form on their own could schedule pre-screening visits (SV0) in order to provide assistance with these and possibly other forms. The pre-screening visits (SV0) may be scheduled for other reasons (e.g., to more fully explain the study prior to ascertaining interest in various program components) as a clinic option. A self-administered version of the eligibility screen will also be available for clinics that choose to obtain eligibility information by mail or in a pre-screening clinic visit. For efficiency, pre-screening visits would likely be conducted in a group setting.

Prior to a woman's SV1, she should be classified as continuing eligible for HRT, DM, or both. First screening visits will not typically be conducted for women known to be ineligible for both the HRT and DM components unless needed to meet OS recruitment goals.

Figure 2
WHI's Model Enrollment Activities and Flow:
Pre-screening Through First Screening Visit



5.2.3 First Screening Visit (SV1)

At SV1, women will first be given a general description of the WHI study, and consent will be obtained to cover SV1 activities. A general medical information release form will be signed at this time. Women who have not completed a Personal Information Form or FFQ will do so at this time. Completed forms and eligibility information will be reviewed and the FFQ will be scanned. Clinic personnel will record each medication and each vitamin or mineral supplement bottle a woman brings; and will conduct a brief in-person exogenous hormone usage interview. Note that women interested in HRT who have recently been on hormones must have discontinued hormone use at least three months prior to this visit. All baseline measures except the Eligibility Screen and FFQ must be completed after the 3-month washout.

Women will then undergo limited screening measurements including pulse, systolic blood pressure, diastolic blood pressure, waist and hip circumference measures, and height and weight measurements. If they are fasting, blood will be drawn. The blood tube to be sent to a local lab will not require processing but must be refrigerated and delivered to the local lab within 12 hours. The blood to be centrally stored will be centrifuged and aliquoted, and frozen to -70 C° for forwarding to the specimen repository. Blood pressure should be measured before the blood draw is done. Women at the three selected Osteoporosis CCs will also provide a urine sample and will be referred for bone densitometry.

By this time, the completed FFQ should have been scanned and analyzed and a determination of FFQ eligibility for the DM component made. This, along with physical measurements, will permit an updated assessment of continuing eligibility for the HRT and DM components. Eligible women will then be given an in-depth description of the pertinent CT components and will be asked to indicate which, if any, CT components they are willing to enter. Those indicating willingness for one or both CT components will be provided pertinent consent forms as well as Medical History, Reproductive History, and Psychosocial questionnaires to complete and return at SV2. As a local option, these forms could be filled out in clinic and may be administered any time before randomization.

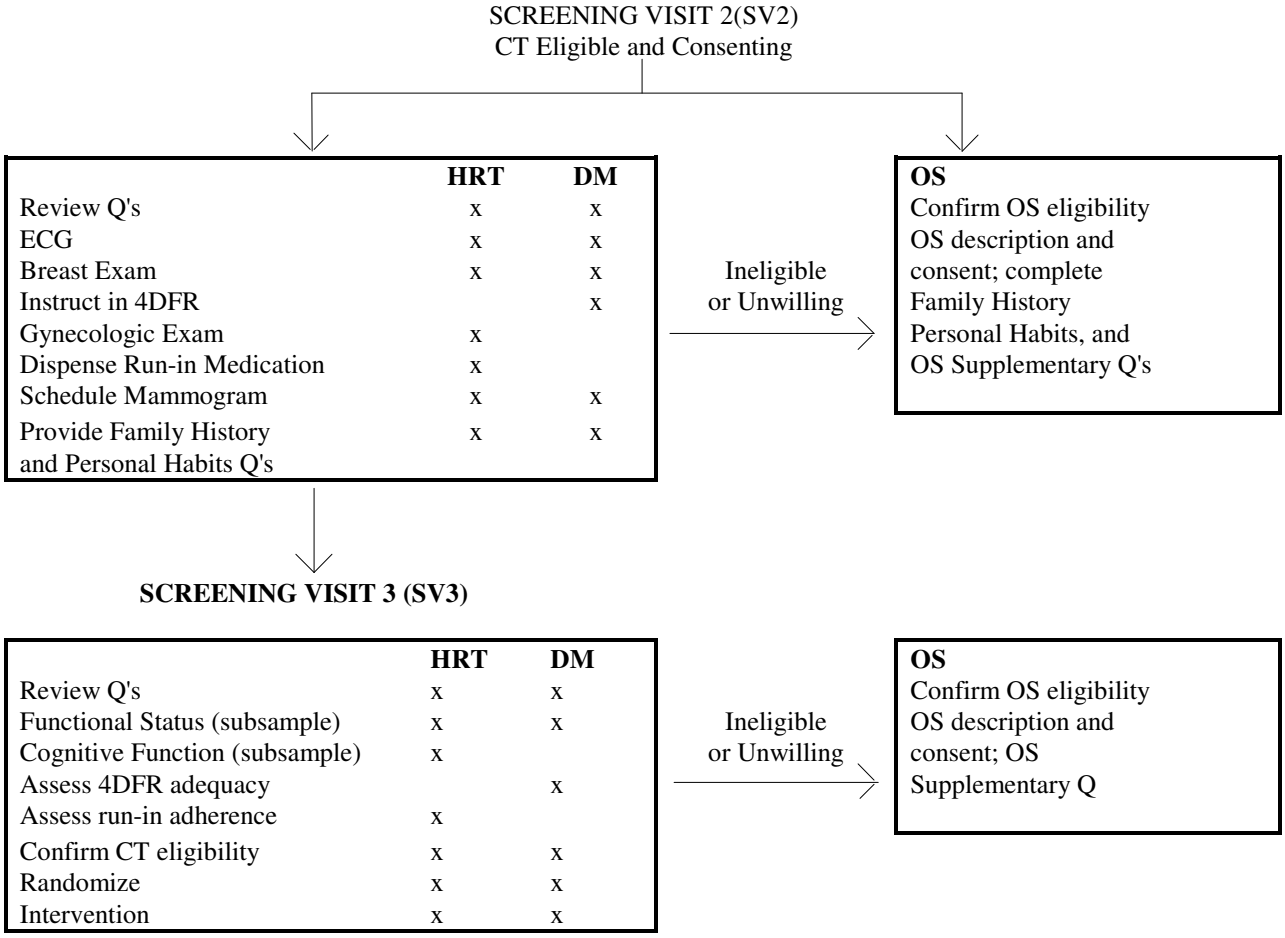
An SV2 will be scheduled as soon as possible after SV1, allowing sufficient time to obtain local laboratory results. If blood was not drawn at SV1, arrangements for a fasting blood draw will be made on or before SV2.

Women who prove to be ineligible for either the HRT or the DM components at the time of eligibility updating, or women who subsequently decide that they are unwilling to be enrolled in the CT, will be invited to consider OS enrollment. As shown in *Figure 2*, such women will then be provided an OS description and will be asked to sign an OS consent form. They will be asked to complete Medical History, Family History, Reproductive History, Personal Habits, Psychosocial and Supplementary OS questionnaires in order to complete their baseline OS requirements. As a clinic option, some or all of these forms may be sent home with the participant for completion and return to the clinic within a 2-week period.

The OS participants will be asked to keep the CC abreast of any change in address and will be told to expect to be contacted in three years for a follow-up visit, once yearly by means of a newsletter, and once yearly near the anniversary of their enrollment for completion of some self-administered questionnaires. They will be thanked for their participation and the visit will be closed.

Potential OS participants who have not provided a fasting blood sample (or urine sample, if appropriate) will have a clinic visit scheduled, preferably within the subsequent two weeks, for the provision of such a sample. OS enrollment will not be effected until all baseline information and specimens have been obtained.

Figure 3
WHI's Model Enrollment Activities and Flow:
Second and Third Screening Visits



5.2.4 Second Screening Visit (SV2)

The SV2 is designed around the medical procedures required for CT participants (see *Section 1-A1, Protocol Appendix 1*). Attempts should be made to complete and evaluate all SV1 activities prior to a woman's second visit. Women who are found to be ineligible in the interim should be notified and invited to join the OS. Those agreeing will have a clinic visit scheduled to afford completion of OS baseline activities as shown in *Figure 3*. Alternatively, if specimen collection and physical measures have been completed, OS consent and outstanding questionnaire information may be obtained by mail, followed by OS enrollment.

At the beginning of SV2 each woman will be given an opportunity to ask additional questions about the CT and the informed consent. Women who are still interested will be asked to sign the informed consent for each component to be entered. The Medical History, Reproductive History, and Psychosocial questionnaires will be collected and reviewed if they have not been reviewed previously.

Women with continued eligibility for the CT will have a resting 12-lead electrocardiogram. The electrocardiogram and complete blood count reports will be reviewed by the clinic practitioner (registered nurse, nurse practitioner, physician assistant, or physician), who will also perform a clinical breast exam and provide breast self-examination instruction.

Women who have had a mammogram within 12 months of SV2 will be asked the name of the mammographer and facility so that results can be obtained. If more than 12 months have elapsed since the last mammogram, a mammogram will be scheduled.

During this part of the second visit, all potential participants of the HRT component will receive a pelvic exam and Pap smear. Those women without prior hysterectomy will also have an endometrial aspiration. Women who have had a Pap smear, endometrial biopsy (or diagnostic D&C) within 12 months prior to SV1 may not need to have these tests at baseline. Women for whom an endometrial biopsy was not successful due to cervical stenosis will have a transvaginal uterine ultrasound as their baseline endometrial evaluation.

Women eligible for, and planning to enroll in, the HRT component, will receive the run-in placebo tablets dispensed in a bottle containing 50 tablets. WHI tablet dispensers should also be given at this time. The potential HRT participants will be instructed carefully regarding steps they should take should they experience vaginal bleeding and will be instructed on keeping a HRT Calendar that they will bring with them to Screening Visit 3 (SV3).

For those women wanting to enroll in the DM component, training in completing the Four Day Food Record will be given with the help of an instructional video. Potential DM participants will then be given time to practice recording a meal, and the dates for completion of the four day food record will be assigned.

A third clinic visit will be scheduled for all women interested in and eligible for either or both the HRT and DM components. To ensure mammography and gynecologic pathology results are available, up to six weeks should be allowed between Screening Visits 2 and 3. The minimum interval needed for the HRT run-in is four weeks. These women will be provided with Family History and Personal Habits questionnaires to complete and return at SV3.

Women who do not provide CT consent, or who are found to be ineligible or unwilling during the course of SV2 will be invited to join the OS. As shown in *Figure 3*, consenting women will be asked to complete the remainder of the OS baseline activities as were detailed in the SV1 description.

5.2.5 Third Screening Visit (SV3)

Final evaluation of CT eligibility and subsequent randomization are the primary activities of SV3. All SV1 and SV2 activities should be completed and evaluated prior to a woman's third visit. Women found to be ineligible between SV2 and SV3 will be notified and invited to join the OS. Those agreeing will have a clinic visit scheduled to allow completion of OS activities. If specimen collection and physical measures have been completed, OS consent and outstanding questionnaire information may be obtained by mail, followed by OS enrollment.

At the beginning of SV3, the Family History and Personal Habits questionnaires will be reviewed. Other SV3 activities are specific to the CT components in which the woman intends to participate. Women planning to enter both the HRT and DM components must complete both sets of activities as described below. The two randomizations for women entering both the HRT and DM components should be conducted on the same day.

1. HRT

   Before SV3, the clinic practitioner will review the results of the mammogram, the Pap smear and, for non-hysterectomized women, the endometrial aspiration. The women in the HRT component will be asked to bring their WHI tablet bottles and dispensers with any remaining tablets, to this visit. When the women arrive at the clinic, medication adherence will be assessed and their HRT Calendar will be reviewed. HRT-only women judged ineligible or declaring themselves unwilling to be randomized will be invited to participate in the OS and, if interested, will complete OS activities as shown in *Figure 3*. Women with any abnormal mammogram, Pap smear, or endometrial biopsy results will be referred back to their primary physician for further evaluation.

Women still eligible for the HRT component will be randomized as described in *Protocol Section 5.2.6* and instructed in the use of medications, and their first 6-month supply of tablets will be dispensed. They will be instructed carefully regarding steps to take should they experience vaginal bleeding and will be instructed on keeping the HRT Calendar that they will bring with them for the next two visits. They will also be asked to contact their CC should they see a physician or be hospitalized for any of the relevant potential adverse effects including breast or endometrial cancer, endometrial hyperplasia, or hysterectomy. All HRT participants will be given a randomization packet containing general health information and written material describing their role in the trial.

2. DM

Women interested in the DM component will have completed their Four-Day Food Records and will bring them to this visit for review according to completeness criteria. Their mammogram reports will be reviewed and final eligibility determined. Those still eligible for this component will be randomized at this time. Women assigned to the DM control group will have the importance of their role described and emphasized. Enrollees assigned to the DM intervention will be assigned to a dietary intervention group. All DM participants will receive a randomization packet that includes general health information and written material describing their role in the trial. DM-only women who became ineligible or unwilling to participate in the DM component will be invited to participate in the OS and, if interested, will complete OS activities, as shown in *Figure 3*.

Cognitive function and functional status measures will be completed on subsamples of CT women (cognitive function 100% of HRT women age 65 and older; functional status 25% of CT women age 65 and older). Clinical Centers may choose to conduct these assessments before randomization.

5.2.6 Study Registration and Randomization

Women who express interest in the DM component, the HRT component, or both will be screened to assess their eligibility for the designated component(s) and all necessary data will be entered into the clinic database during the time between Screening Visits 1 and 3. In each case, informed consent will be obtained according to relevant institutional and legal requirements and recorded in the database. When a woman has completed the necessary screening and provided consent, the data coordinator or designated clinic staff person will execute a database function that will verify eligibility for the designated component(s), assign the woman to a trial arm according to the algorithm described below, determine membership(s) in appropriate subsamples, and produce a confirmation of randomization report. Once a woman has been randomized into a trial arm, she will be followed in that arm regardless of her adherence to her assigned treatment.

To reduce potential contamination in the DM component certain natural groupings of women (particularly those residing in the same household) who are both interested in and eligible for the DM will be randomized as a group to the same trial arm. See *Vol. 2 - Procedures* for approved group formation and randomization procedures.

Women who participate in the DM component, the HRT component or both will be mailed descriptive information on the CaD trial component prior to their first annual visit*. Those expressing interest will have CaD eligibility assessed at their first annual visit, with randomization to occur within an 8-week window surrounding the anniversary of the woman's baseline randomization date.

CT randomization will use a randomized permuted block algorithm, stratified by clinic, age (50-54, 55-59, 60-69, 70-79), and for the HRT, by hysterectomy status. Treatment assignments for all participants in each CT will be generated in the proportions described in *Figure 1*. Block size will be allowed to vary randomly to further preclude any exercise in judgment in the assignment of participants to trial arms. Enrollment into certain cells (e.g., younger ages or post-hysterectomy women) may be closed from time to time in order to meet the design criteria for distributions on these key factors.

* (or through the time of their second annual visit in selected cases)

Observational Study

Women attending at least one clinic visit who are not eligible for or willing to participate in either the DM or the HRT components will be offered the opportunity to participate in the OS. Informed consent will be obtained, additional OS data will be collected and all necessary data entered into the clinic database. When this has been completed (including the provision of a blood specimen and, if appropriate, a urine specimen), the data coordinator will execute a database function that will register the woman in the OS, determine membership in appropriate subsamples, and generate a registration confirmation report.

Sampling for Substudies

Some intermediate effects of trial interventions and measures of adherence and secular trends will be assessed in a subsample of study participants. To account for the different sample sizes for CT components, a cohort of 8.6% of HRT participants and 4.3% of DM participants will be selected at the time of randomization. These subsamples will be stratified by clinic, age, race, and hysterectomy status in a manner that achieves equity in CC burden while preserving the ability to address questions of interest, particularly intermediate effects, in minority populations. Laboratory measures, completion and documentation of Four-Day Food Records, collection of 24-Hour Dietary recalls and repeated assessment of quality of life will be performed on these cohorts. All HRT women 65 years of age and older will be selected for cognitive function assessment at baseline and follow-up. Another 25% sample of CT women 65 years of age and older will be selected for baseline and follow-up measures of functional status. Additional subgroups will be randomly selected using repeated cross-sectional sampling for other selected measures (e.g., FFQ, 24 hour dietary recall, endometrial aspirations in PERT and placebo arms). A 1% subsample of OS enrollees will be selected for reliability studies to be conducted at baseline and year 3. Details of sampling schemes for other studies can be found in *Vol. 1 - Study Protocol and Policies*.

5.3 Follow-Up

5.3.1 Clinical Trial

1. General CT Follow-Up

   Clinical Trial participants will be followed through regularly scheduled examinations to collect data on study variables, to monitor the occurrence of possible adverse effects, and to promote adherence to study protocol. Annual visits will be scheduled within the four-week interval surrounding the anniversary of their randomization into the CT. All CT participants will have interim six-month contacts, either by phone, mail or visit, at clinic option, to obtain the participant's updated medical history. Clinic visits will be conducted at six months following enrollment into the HRT component. In the event that the annual or six-month contact cannot be conducted within the target time interval, such a visit will be conducted as close as possible to the time window. All participant data will be entered into the database. The required procedures and data to be collected at each visit are specified in *Appendix 1*.

   Before each annual visit, all CT participants will be mailed the Medical History Update and other questionnaires (including the occurrence of any outcomes of interest) to be completed at home and brought with them to their clinic appointment. At years 1, 3, 6 and 9, they will also be asked to bring in all their medications and vitamin supplements for an updated inventory. At the annual visit, all CT participants will have their questionnaires reviewed for potential outcomes. In addition, CT participants will have a brief physical exam. ECGs will be obtained at years 3, 6 and 9. Additional measures will be obtained at the first annual visit and at years 3, 6, and 9, with some elements restricted to a subsample. If the participant has any concerns or symptoms, she will have an opportunity to discuss them with a clinic practitioner. If any potential outcomes are reported, the clinic will initiate the appropriate ascertainment and classification protocol. An appointment may be made for the participant's next visit.

At least six weeks prior to each HRT participant's anniversary of her most recent mammogram, the CC will request that the woman have a mammogram, thereby ensuring that the results will be available at the annual clinic visit. For DM participants not randomized into HRT, a mammogram will be required every second year using the same procedure.

At the first annual visit all CT participants will have their blood drawn and stored. Blood will be drawn and stored on a subsample of women at the third annual visit and at every subsequent third annual visit. For women randomized at clinics participating in the osteoporosis substudy, bone densitometry studies and urine collection will be done at the first and third annual visit and every three years thereafter.

Certain behavioral questionnaires will be re-applied to all CT participants at their first annual visit and in a subsample at 3, 6, and 9 years.

2. Hormone Replacement Component Specific Follow-Up

At approximately six weeks after randomization, HRT participants will be contacted by phone by CC personnel in order to answer questions the participant may have and to identify any major adverse experiences that have not been self-reported. For safety reasons HRT participants will be scheduled for 6-month interim contacts. The first 6-month interim contact will be a clinic visit. Subsequent interim 6-month contacts may be by either mail, phone, or visit, as determined by the CC.

At both the first 6-month semi-annual and all annual contacts, HRT participants will be asked to return their unused HRT tablets and their adherence will be assessed by measuring remaining tablets. During the first year, their HRT calendars will be reviewed by the designated clinic contact. A brief questionnaire will be administered to each participant to identify potential adverse effects and adherence problems. If there has been any bleeding, or if the participant has had any adverse effects other than minor symptoms, the participant will be seen by the designated clinic contact and the bleeding/adverse effects will be reviewed. The contact may decide at this time that other work-up or referral is necessary. After the participant is cleared, she will be dispensed a new supply of tablets and a new HRT Calendar (first semi-annual visit only).

For HRT participants with a uterus the annual physical exam will include a pelvic exam (performed by the WHI clinic medical staff or the participant's personal physician). Every three years, HRT participants will have a Pap smear, either through the CC or their personal physician. On a 5-6% random subsample, the WHI clinic practitioner or gynecologist will perform endometrial aspirations in years 3, 6, and 9. A transvaginal ultrasound will be performed if an endometrial aspiration proves impractical (see *Protocol Section 5.5.2.2*).

On July 9, 2002, after an average follow-up of 5.2 years, the intervention phase of the PERT (Estrogen plus Progestin) trial ended and Estrogen plus Progestin participants were told to stop their Estrogen plus Progestin study pills (see *Section 4.7.1. – Hormone Replacement Therapy*) and asked to complete a current medical history update. During the subsequent three months, these participants were unblinded to their treatment arm. Follow-up of the Estrogen plus Progestin participants continues on a semi-annual basis with the following changes: HRT safety interviews will be completed for the next two semi-annual contacts after stopping study pills. Pelvic exams and Pap smears are no longer required for Estrogen plus Progestin participants, but may be continued for retention purposes at CC option. The 5-6% random subsample endometrial aspirations are no longer required. Follow-up of ERT (Estrogen-Alone) participants continues as before.

3. Dietary Modification Component Specific Follow-Up

An FFQ will be collected at year 1 on all DM participants and in a subsample of DM participants in the remaining years. The subsample of women selected at randomization will be asked to complete a Four-Day Food Record at year 1 and multiple unannounced 24 hour dietary recalls at years, 3, 6 and 9. An additional small independent sample (1%) will be chosen for unannounced 24-hour dietary recalls at selected time points.

4. Calcium/Vitamin D Trial Component Specific Follow-Up

   At approximately four weeks after randomization, CaD participants will be contacted by phone by CC personnel in order to answer questions the participant may have and to identify any major adverse experiences that have not been self-reported. Any difficulties in taking the pill of choice (either chewable or swallowable) should be addressed and options discussed. Thereafter their follow-up schedules are determined by their involvement in the other CT components. Women participating in the CaD component will be asked to return their unused tablets at follow-up visits. Adherence will be measured by measuring remaining tablets. Unless contraindicated by a report of renal calculi or other adverse experiences as described in *Protocol Section 5.5*, a new supply of tablets will be dispensed.

5.3.2 Observational Study

At the time of enrollment, a 1% sample of OS participants will be selected to have a repeat visit between one and three months of their enrollment visit and again after their 3-year visit. The measures and specimens collected at these visits will be those thought to be subject to noteworthy measurement error (e.g., laboratory measures, physical activity).

Routine follow-up for OS participants consists of mailed newsletters and self-administered questionnaires and limited clinic visits. Additional measures may be incorporated through the ancillary study mechanism.

Before the anniversary of their enrollment in the OS, OS participants will be mailed a self-administered Medical History Update questionnaire, an OS Exposure Update questionnaire and a postage prepaid return envelope. A sequence of contact attempts involving both CCC and CC efforts will be implemented to assure the follow-up goals are met. For the first annual follow-up the goals are: 1) to ascertain vital status on 99% of OS participants; 2) to have completed Medical History Update questionnaires on 95% of participants; and 3) to have OS Exposure Update completed by 90% of participants.

OS participants will also be mailed an annual newsletter prepared by the CCC at about six months following their OS enrollment or their OS enrollment anniversary. The purpose of the newsletter is to further bond participants to the study and to obtain updated addresses.

Three years after enrollment into the study, all OS participants will be invited to a follow-up clinic visit. Before this visit they will be mailed a packet of questionnaires that will include questions on health habits, medical history and outcomes, as well as psychosocial and food frequency questionnaires. They will be asked to bring in their current medications and supplements. Participants will have the option of completing forms at the clinic. At the clinic visit, they will have blood drawn, their medications and supplements will be recorded and the following measurements will be taken: height, weight, waist and hip measurements, and blood pressure. At the three osteoporosis substudy centers, bone densitometry studies and urine samples will be completed for all OS women every three years. If no response is received to the 3-year visit invitation, every effort will be made to contact the participant by phone and to schedule a clinic visit.

5.3.3 Study Close-out

Assuming that one or more CT components are not terminated early, planning for close-out will begin four years prior to the actual close-out year. This will begin first with the formation of a close-out committee, consisting of representatives from NIH, the CCC, and the CCs. This committee will consider the issues involved in the scheduled termination of clinical activities, and will present detailed plans to the Steering Committee. During the three years prior to the close-out year, efforts will be intensified to locate lost-to-follow-up participants at the time of their anniversary date and last scheduled follow-up visit.

CT participants who attend the annual visit prior to their final close-out visit will be notified that the study termination is approaching, and efforts to lessen the psychological effects of study termination will be initiated. Six months before their close-out visit, participants will be sent literature about the study close-out, and reminded to expect their close-out visit. The close-out contacts will consist of a visit around the calendar time of the annual visit, followed by a phone contact six weeks later.

The close-out visit will have many of the same elements as an annual visit, that is, participants will be mailed questionnaires, including the Medical History Update to complete and bring to the visit. They will be asked to bring in their study medications for measurement. They will also bring in their current prescription medications, currently used over-the-counter medications and supplements, for updating the medication and supplements inventory. The usual anthropometric measurements will be taken. Women with uteri in the HRT component will have a pelvic exam and Pap smear. All CT women will have a mammogram (unless contraindicated), breast exam, and ECG. A detailed personal data form will be completed at this time, so that all contact information can be updated. In addition, all CT women will also be informed as to the schedule and nature of information that they will receive concerning principal results from the trial. Additional close-out procedures for each specific CT component will be as follows:

1. HRT

   Both participants and providers will continue to be blinded at the close-out visit. Study medications will be discontinued, and participants will be carefully instructed regarding symptoms they might expect from discontinuation of hormones. A list of common symptoms and suggested steps to alleviate symptoms will be provided. Participants will be advised to call the clinic practitioner if they have any severe symptoms, or any significant vaginal bleeding. It may be necessary for the designated clinic contact to consult with the consulting gynecologist before the next scheduled contact, if it appears that a participant will require ongoing HRT. In this case, the designated clinic contact will contact the participant's primary physician, and make arrangements for the participant to be treated by the primary physician, after speaking with the clinic consulting gynecologist.

   Participants will also fill out a form to record their best guess of what treatment group they were assigned to, to assess the degree of the double-blind. A form will also be completed to ascertain if the participant has a continuing source of medical care and whether all study medications have been returned.

   A close-out telephone contact will take place approximately six weeks after the close-out visit. This may be a clinic visit for participants without phones. Both participants and clinic staff will be unblinded at this time. At this contact, participants will be asked questions regarding symptoms since discontinuing study medications. Questions to be addressed will include:

   - Whether symptoms thought to be associated with study drugs have been relieved once medication was discontinued.
   - Whether new problems have arisen that could be associated with stopping study medications.
   - Whether the participant has been prescribed hormones by an outside physician since her close-out visit.

2. DM

   Participants in the DM component will be told that the formal trial will end. The intervention group participants will be told that dietary supervision and meetings arranged by clinic staff will end but they will be given every possible assistance to maintain their low-fat, high fruit and vegetable eating patterns and to arrange their own meetings before their final visits. Women in the control group will be told that official dietary intervention for the other group will end and that control group participants will be given an opportunity to attend one or two special instructional sessions on ways to lower the fat in their diets and will be offered self-help materials to help them to modify their eating patterns. These materials will be based on the most successful components of the WHI intervention. Any new dietary advice that becomes available during the conduct of the trial will be shared with both groups of women.

3. CaD

   Women in the CaD component will be participants in at least one of the two other clinical trials, and the measurements taken during their close-out visit will follow those for the HRT and DM components. At the close-out visit their adherence to study medications will be assessed by tablet measurement, and their drugs will be discontinued. Participants will be asked to provide their best guess of their treatment

assignment. It is unlikely that they will experience any significant symptoms from stopping treatment, and they will be informed of this. At this contact, forms will be completed to document participants' continuing source of medical care, and to document that study drugs have been returned. At a close-out phone contact six weeks later, participants and clinics will be unblinded. If participants on the active CaD arm choose to continue treatment, drug and dosage information will be provided to them and their primary physician.

4. Training and Trial Documentation

   Training will be provided for CC staff to counsel CT participants as they exit from the study. At the conclusion of the CT, each participant will be provided with a summary of overall trial results as appropriate and selected results from their clinical exams and laboratory tests. The CCC, in accordance with guidelines developed by the Project Office and the Council, will prepare and document the final database.

5. OS

   Two months prior to study close-out, participants in the OS will receive notification by mail that the study is coming to a close. At this time they will be sent a final medical history update questionnaire to complete and return to the CC. They will also be sent literature about the study close-out.

5.4 Adherence and Retention

Retention of study participants and their adherence with the study protocol is a dominant focus after the participant is enrolled. Retention has several components: Adherence (taking study drugs), Performance (maintaining low-fat dietary consumption), and Participation (attending follow-up visits, and accepting telephone calls). The evidence from randomized evaluations and evidence from observational studies of participant accrual and follow-up suggests that personal attention from study staff and specific and reassuring feedback about required follow-up activities are themselves useful retention strategies. Correlational evidence indicates that freedom from worries about health, comfort with the intervention materials, and higher SES are related to retention. Taken together, these studies suggest that a retention protocol that will increase social support and positive interactions while minimizing unnecessary health concerns and worry, will maximize retention in WHI.

The CCC will provide each CC with a package of core study-wide retention enhancements. Personal contacts, visits, and follow-up phone calls will be the cornerstone of CC-specific retention efforts, while making sure to avoid the introduction of any contamination or bias. The CCC will coordinate scripts and provide interviewer and staff training and guidelines for standardized contacts using social support and health-related messages. Each CC will continue to implement its own local additional retention efforts to complement study-wide functions. The following strategies exemplify those that may be included:

- Appointment reminders (postcards and telephone contacts)
- Newsletters
- Methods for involving family members
- Special events
- Local Participant Ombudsman
- Modest incentives (magnet, pins, mugs, calendars, etc.)
- Health-related informational materials
- Weekly tablet dispenser
- Physician letters

5.5 Evaluation and Management of Adverse Experiences in the CT

5.5.1 Adverse Experience Monitoring

When informed consent is obtained, potential adverse effects of study treatments will be explained to each prospective participant. Written material outlining these adverse effects will be provided and the women will be instructed to notify the CC of any adverse experiences, illnesses or hospitalizations. Data on adverse experiences will be entered in the database and reported regularly through the processing of outcomes. The Food and Drug Administration granted a waiver of standard Investigational New Drug Serious Adverse Experiences report. These adverse experiences will be collected and reported under all circumstances and without the assumption that they are related to study treatment. Participants will be appropriately monitored until the end of the trial. Copies of the documentation that led to the identification of an adverse experience will be archived at the CC for at least two years after termination of the study.

The Data and Safety Monitoring Board (*Protocol Section 10*) will periodically monitor a range of potential side effects and make appropriate recommendations to ensure participant safety.

5.5.2 HRT

5.5.2.1 General

Prior to randomization, HRT participants will be briefed on the possible side effects from the study drugs and the medical significance of these possible side effects. Written material outlining these adverse effects will be provided. During the first year participants will be instructed to record any vaginal bleeding or spotting in their HRT Calendar and to notify the designated clinic contact (see *Protocol Section 4.7*) at the time vaginal bleeding first occurs. Routine endometrial aspiration biopsies will be performed prior to randomization and in an appropriate subsample of HRT participants, at regularly scheduled intervals during the trial. Diagnostic endometrial evaluation will be performed at the request of the clinic consulting gynecologist.

5.5.2.2 Endometrial Evaluation

Routine endometrial evaluation will be performed in all women with a uterus at baseline, and in a random 5-6% subsample in years 3, 6, and 9. Women with abnormal baseline biopsies will be excluded from HRT.

Diagnostic endometrial evaluation will be performed at the request of the clinic consulting gynecologist, who will maintain a copy of all records concerning vaginal bleeding and baseline, follow-up and diagnostic endometrial evaluations. Appropriate clinic staff will record this information on the study forms. All follow-up endometrial biopsy samplings will be evaluated locally for therapeutic decision-making. A standardized classification system will be used.

All endometrial biopsies (routine or diagnostic) will be performed with a flexible aspirator device. Entry into the uterus, by definition, will indicate a successful procedure, regardless of whether or not adequate tissue is obtained. If the uterus cannot be entered with the flexible aspirator device, a second attempt will be made by a different operator, using cervical block anesthesia. If these two attempts fail at passing the cervical OS, a transvaginal uterine ultrasound will be performed.

Normal endometrium refers to any pathologic finding from tissue biopsy that is compatible with atrophic, proliferative or secretory endometrium. Insufficient tissue obtained for diagnosis also qualifies as normal endometrium. Other biopsy findings require evaluation and management or referral to the primary physician by the clinic gynecologist. Endometrium with a thickness $\leq$ 5 mm on transvaginal uterine ultrasound is considered normal.

Abnormal endometrial findings refer to:

- Simple hyperplasia

or

- Adenomatous, complex or atypical hyperplasia or endometrial cancer

If the transvaginal uterine ultrasound shows a thickness of the endometrium > 5 mm, the participant will be referred to her primary physician for further evaluation.

5.5.2.3 Management of Vaginal Bleeding

CCs should do an endometrial aspiration on those Estrogen plus Progestin participants who stopped intervention in July 2002 and present with spotting or bleeding more than 8 weeks after stopping study pills. Participants may elect to have this procedure done by their outside provider. Any abnormal endometrial findings (as defined in Section 5.5.2.2. – Endometrial Evaluation) will require follow-up and management by an outside provider.

5.5.2.4 Discontinuation of HRT Treatment*

If a woman develops breast cancer, deep vein thrombosis, pulmonary embolus, malignant melanoma, or triglycerides >1000 mg/dl, her Estrogen-Alone study pills will be permanently discontinued without unblinding.

Study pills will also be discontinued (without unblinding) if the participant's physician prescribes estrogen, progesterone, testosterone, Tamoxifen, or other selective estrogen receptor modulators.

Refusal of a routine post-randomization mammogram within 18 months of her previous mammogram will result in discontinuation of Estrogen-Alone study pills with continued follow-up of the woman. See Vol. 2, Section 16.4.2.1 – HRT (Minimum Procedures for a CT Participant to Remain on Intervention) for other minimum safety requirments. If the participant later agrees to the procedure, study pills will be resumed.

In addition, Estrogen-Alone study pills will be discontinued temporarily if any of the following experiences occur:

- Myocardial infarction;
- Stroke;
- Surgery involving the use of anethesia;
- Any fracture or major injury involving hospitalization;
- Any illness that results in immobilization for more than one week; or
- Any severe illness in which HRT is temporarily inappropriate (including newly diagnosed TIAs or other cardiovascular conditions that may increase a participant's risk of a thrombotic event).

Finally, women who have intolerable symptoms refractory to conservative therapy according to a step-down protocol described in *Vol. 2 - Procedures, Section 5.4.1.4 - Step-Down Dose Management for Refractory Symptoms* may need to stop their study pills.

5.5.2.5 Changing the HRT Arm

Women who were randomized to the PERT arm but who subsequently had a hysterectomy for reasons other than cancer were eligible to continue on study pills. Since there is no routine clinical indication for women without a uterus to be given progestins, women who had a hysterectomy during the trial follow-up (before the intervention was stopped in July 2002) were changed from PERT to ERT. They will be followed in the PERT arm for outcomes, however.

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* The Estrogen plus Progestin intervention was stopped for all PERT participants in July 2002. *Discontinuation of HRT Treatment* refers only to participants in the Estrogen-Alone (ERT) trial.

All changes in hormone use will be documented in the study database.

5.5.3 Dietary Modification Component

Any experiences that require a special diet may result in the temporary or permanent discontinuation of the dietary intervention, including:

- Newly developed Type I (ketosis prone) insulin-requiring diabetes
- Gastrointestinal disease or surgery, such as malabsorption syndrome, short gut syndrome, etc.
- Acute or chronic pancreatitis

Such discontinuation will be decided by the clinic physician in conjunction with the participant's primary physician and notification will be sent to the CCC. All such women will continue to be followed for outcomes in their assigned randomization group.

5.5.4 Calcium/Vitamin D Component

Women who develop renal calculi or hypercalcemia or those requiring kidney dialysis will have CaD pills permanently discontinued. Women taking Calcitriol or > 1,000 IU of personal vitamin D supplements will have CaD pills discontinued while on these therapies.

The following adverse experiences may result in the temporary discontinuation of the CaD therapy:

- Any hospitalization
- Accidents resulting in immobilization
- Myocardial infarction
- Stroke
- Any severe illness in which the administration of CaD is temporarily inappropriate

5.5.5 Notifications

5.5.5.1 Immediate and Urgent Referrals

The clinic physician or practitioner will need to evaluate the urgent referrals to determine if it is necessary to move a referral from urgent to immediate. Immediate referrals are medical emergencies which require immediate notification of both the participant and her primary physician. Immediate notification of the participant should occur during the clinic visit. Immediate notification of the participant's physician should be accomplished by telephone, to be completed before the participant leaves the CC. A follow-up letter documenting information discussed by phone should also be sent to the participant's physician. Findings requiring immediate referral are as follows (clinics may define additional referral criteria at their discretion):

- Medical History:
  - Severe depression
- Physical Examination:
  - Any problem the CC physician feels requires attention immediately (for example, exacerbation of congestive heart failure, acute asthma episode, BP > 210/120 mmHg, serious arrhythmia, etc.)
- Electrocardiogram (CT only):
  - Acute myocardial ischemia/injury
  - Sustained ventricular tachycardia

Urgent referrals are made for abnormalities detected which require medical attention but not on an emergency basis. Urgent notification of the participant should occur before the participant leaves the CC, or immediately upon receipt of the finding from the local laboratory or central pathologist. Urgent notification of the participant's physician should be sent within the week. Findings requiring urgent referral are as follows:

- Medical History:
  - Severe vaginal bleeding*
- Physical Examination:
  - Resting pulse rate < 40/min or > 130/min
  - DBP > 105 mm Hg
  - Suspicious breast mass
  - Pelvic mass
- Endometrial Evaluation:
  - Cancer or atypia*
- Mammography:
  - Finding suspicious for cancer
- Electrocardiogram (CT only):
  - Atrial flutter or fibrillation (new onset)
  - Mobitz type II AV block
- Hematology
  - Hematocrit < 30% or hemoglobin < 10 gm/dl
  - WBC < 1,000 cells/mm3
  - Platelet count < 50,000 cells/ml

**Note:* Asterisks (*) in the above listing imply that the finding is evaluated and referred by the clinic gynecologist.

5.5.5.2 Routine Referrals

Physical findings and laboratory values as well as copies of electrocardiograms and Pap smear, endometrial aspiration and mammography reports could be sent routinely to participant's physicians (or given to participant to bring to her physician). Decisions regarding specific reporting should be made at the CC level in the context of community referral practices and participant's preferences. Participants will be asked at the beginning of the study for their permission to send such reports to their physician.

The following findings may be considered for reporting if measures are available:

Medical History:

- Unexplained weight loss
- Cognitive decline
- Angina (new or uncontrolled)

Physical Examination:

- Blood pressure
- Weight

Bone Densitometry:

- Baseline bone mineral density at hip more than 3 standard deviations below mean for age
- Rapid bone loss (>10% per year, or >20% over 3 years)

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6. Centralized Study Operations

6.1 Data Management

All routine data will be collected and entered by the CCs using certified data collection staff and data collection forms or direct entry screens provided by the CCC. For data and clinic management purposes, each CC will be equipped with a local area network (LAN) consisting of a Novell file server, five personal computers (PC), a printer, two barcode readers, and a mark-sense reader (scanner). Each file server will be loaded with software for the following functions: network management graphical user interface (Windows), data management (developed by the CCC in Oracle), word processing (Word for Windows), spreadsheet (Excel for Windows), and electronic mail. Each CC PC will be connected to the file server through the LAN in order to provide shared access to clinic data and software. Each CC LAN will be connected to the CCC by a wide area network (WAN). The WAN will link all WHI file servers over dedicated communications lines and will provide continuous communication abilities. Most equipment will be delivered directly to the CCs. The file server and WAN equipment will be delivered to the CCC for configuration. The CCC will be responsible for daily incremental back-ups of all study-wide data over the WAN. Additional aspects of the data management system will be specified in *Vol. 5 - Data System*.

6.2 Quality Assurance

The quality of study-wide operations, data, and products will be assured by a variety of methods including clear and complete documentation, centrally and locally managed training and certification, routine reports, quality assurance (QA) site visits and task specific quality assurance measures (e.g., routine observation, chart audits, duplicate data entry) as deemed appropriate by the CCC, the Steering Committee and Project Officer. The training and certification required for each study task is described in *Vol. 2 - Procedures*. In addition the CCC will perform cross-sectional and longitudinal edits of the central database. Data queries resulting from these edits, and from reporting and analysis activities, will be submitted to the CCs for resolution, and a systematic means of updating the central database based on their responses will be established. To assist in addressing these queries, the CCs will be required to store hard copies of their data collection forms in a readily accessible manner, and to respond to queries in a timely fashion. Standards for performance are proposed by the CCC (see *Protocol Section 9*), approved by the Steering Committee, and documented in the WHI Manuals. Study units determined to be operating below acceptable performance levels will be required to submit plans for remedial action to the Steering Committee for approval and will be subject to more intensive monitoring.

6.3 Drug Distribution

Study medications will be shipped to the CCs on a regular basis by the CCC drug distribution center located at McKesson BioServices. Study medications will come in several forms: placebos for the run-in period of the HRT; blinded medications for the HRT (Placebos, ERT and PERT); open label conjugated equine estrogen and medroxyprogesterone for management of some adverse and side effects of HRT; and blinded medications for the CaD component. Bottles for the run-in period will contain 50 tablets; for follow-up, HRT bottles will contain 215 tablets. CaD tablets will be dispensed in bottles. All medications will be identified with a unique bottle number for tracking and inventory purposes. CCs will log each incoming shipment and each bottle dispensed or returned into the CC database upon receipt or dispensation. For blinded study medications, the bottle number will be linked to trial arm in the CC database. This link will not be accessible to CC database users. To dispense blinded study medications, the data coordinator or other authorized clinic staff member will execute a database function that will identify an appropriate bottle in the drug inventory at the clinic site. Clinical Centers will be responsible for labeling each bottle with the participant's name, identification number, and CC information.

6.4 Outcome Adjudication

For purposes of attaining high quality outcome data for each CT component, the primary outcome diagnosis will be analyzed by local CC physician representatives. Until strong agreement is assured between local clinical diagnoses and a central determination ($\geq$ 90% agreement on primary diagnosis for CHD and fractures)

the data packets, including discharge summary and respective data reports, will be sent to the CCC for central adjudication. After satisfactory agreement has been demonstrated for a CC in these categories, central adjudication will occur on a sampling basis. For the primary cancer outcomes, all data packets will be submitted to the CCC for coding by a qualified SEER coder. The specifics for each scheme of adjudication within the cardiovascular, cancer and fracture outcomes is detailed in *Volume 8 - Outcomes*. In general, while central adjudication may be part of outcome assignment for each study component in the CT, the expectation is that the central adjudication and quality assurance measures for the CT will be sufficient to assure the quality of local diagnoses for the OS.

7. Study Monitoring and Data Analysis

7.1 General

Progress in the CT and OS will be monitored in several ways: reports on subject accrual, adherence to follow-up procedures, and on intervention adherence rates in the CT will be provided by the CCC to the Steering Committee, as well as to the DSMB and the NIH on a regular basis. Reports on adverse effects and on clinical outcomes by randomization group will be provided on a regular basis to the DSMB. These reports will provide the basis for considerations of remedial actions or protocol changes, and for considerations of early stoppage of CT components.

7.2 Accrual, Adherence and Accumulated Outcome Events

Developing information on subject accrual, and hence average follow-up duration at planned study termination, on adherence, and on the total number of primary outcome events among women randomized to each CT component will be used to produce updated primary outcome power projections, of the type shown in *Section 1-A3, Protocol Appendix 3*. The design assumptions concerning intervention effect on primary outcome rates will be retained in these power calculations, the results of which will be provided annually to the DSMB and the NIH. Remedial action may be indicated if powers (about 90% or greater) under CT or OS design assumptions are projected to fall as low as 80%. Reports on accrual, intervention adherence in the CT, completeness of participation in follow-up and outcome ascertainment activities, and on other aspects of quality assurance, will be provided regularly to the Steering Committee and the NIH for each active CC in order to allow early identification of potential problems.

Accrual information by age, racial/ethnic subgroup, and socioeconomic subgroup will also be monitored in the CT and OS, as will be the fraction of women who are post-hysterectomy in the HRT component of the CT. Noteworthy departures from targeted fractions may give rise to specialized recruitment efforts to recover the desired distributions, or to the temporary closure of some enrollment categories. Adherence in the CT will also be monitored by age, racial/ethnic, and socioeconomic subgroups.

7.3 Monitoring of Clinical Events by Randomization Group in the CT

The development of procedures for monitoring the CT for possible early stoppage poses specific challenges, some of which are unique to the WHI. There is the danger of over-interpreting treatment effects for a CT component early in the trial follow-up period, without adequately acknowledging the fact that multiple outcomes are being monitored and hence chance differences are more probable, and without adequately acknowledging that hypothesized (beneficial or adverse) effects for some outcomes have a substantially later time course than others. Along the same lines there is a danger in over-interpreting a beneficial effect of a treatment on a given CT outcome since the CT treatments have hypothesized benefits and risks for a number of important diseases. For example, early stoppage of the HRT component on the basis of evidence of hip fracture prevention, without definitive data on CHD or breast cancer effects would leave unanswered some of the most important public health issues surrounding HRT. Similarly, if a CT treatment is observed to have both beneficial and adverse effects then trial monitoring procedures need to rely on some suitable composite or summary outcomes, in order that the public health implications be as unequivocal as possible, while simultaneously paying all due attention to the safety of participating women.

To address these issues an independent DSMB for the WHI has been appointed by the NIH Director. Information on the occurrence of outcomes of interest (*Protocol Section 4.3*) by treatment group is presented at regular meetings of the DSMB. Evidence of adverse effects, or of adverse risk to benefit profile, may give rise to recommendations for protocol changes (e.g., concerning dosages or dosage modification procedures in the HRT or CaD components, or concerning dietary goals in the DM component), or in the event of a serious adverse effect or a compelling favorable benefit to risk profile, to a recommendation of early stoppage of a CT component, or of certain treatment arms of a CT component.

The specific procedures for accomplishing such monitoring will be developed in collaboration with the DSMB and detailed in a separate document (see *Vol. 1 - Study Protocol and Policies*). Elements of the plan are

expected to include a reliance on incidence from primary and subsidiary disease outcomes, and on mortality from other causes, with due consideration of the likely time course of various clinical events. In order to allow greater sensitivity to evolving morbidity data, consideration will be given to multivariate comparisons, and to the construction of a composite disease indices that would combine incidence and mortality information from several disease categories. Each such outcome analysis will make appropriate provision for the multiple time points of interim analyses, and for the hypothesized time course of treatment effects in a manner that attempts to avoid premature stoppage while ensuring participant safety.

7.4 Data Analysis

CT

The basic test statistic to be used to compare an intervention group to a corresponding control group, both for CT monitoring and for periodic analysis, will be a weighted (2-sided) log rank statistic. Such a statistic can be written

$$T = \Sigma\, w_i\, (O_i - E_i)$$

where w_i is the value of the weight function evaluated at the ith largest time from randomization to clinical outcome event among women in both groups, O_i is one or zero depending on whether the outcome occurred in a woman in the treated group or not, and E_i is the conditional expected value of O_i. If V_i represents the conditional variance of O_i, then it follows that the variance (s^2) of T is estimated by $s^2 = S\, w_i^2 V_i$ and the test for differences between groups is then made by referring T^2/s^2 to the 95th percentile of a chi-square distribution on one degree of freedom.

The weighting is intended to enhance test power. Since it is anticipated that intervention versus control disease incidence ratios will vary approximately linearly as a function of time since randomization, the weights w_i will be chosen to equal time from randomization up to a disease-specific maximum (three years for cardiovascular disease and fracture occurrence, 10 years for cancer occurrence and total mortality) and to be constant thereafter.

The test statistic will be modified slightly for outcome categories that rely on centralized ECG assessments. Since ECG readings are obtained every three years during follow-up, the test statistic will be replaced by a weighted combination of binomial proportions at three, six, and nine years and at close-out for these outcomes. The weights will be averages of those previously described over the pertinent follow-up period.

In acknowledgment of the partial factorial design the (four) primary outcome tests will be stratified on the categories of the other interventions, baseline age (50-54, 55-59, 60-69, 70-79), and self-reported prevalent disease (if applicable) for that outcome. The primary HRT comparisons will be examined separately based on hysterectomy status. In these and other analyses, the times from randomization to disease occurrence will be censored at the time of death from other disease or loss to follow-up. The primary outcome tests will not be adjusted for multiple testing since each component merits a separate hypothesis test. Corresponding to each of these tests, we will estimate intervention versus control group relative risks as a function of time from randomization using relative risk (Cox) regression methods (Cox, 1972) stratified as just described with suitably defined time-dependent covariates (e.g., Kalbfleisch and Prentice, 1980; Cox and Oakes, 1984). Closely related analyses will also be carried out to estimate a 'full adherence' relative risk function for each intervention in relation to its primary outcome.

The same statistical methods will be used for testing and estimation of the secondary and composite outcomes, as well as the subgroup associations listed in *Protocol Section 3.1*. The same methods will also be used to compare total mortality rates between intervention and control groups. The manner in which these analyses will acknowledge the sequential monitoring aspect of the CT will be described in a separate document, to be developed in conjunction with the DSMB.

More detailed explanatory analyses will include tests for group differences with concomitant adjustment for covariates, as well as explanatory analyses that examine the extent to which an intervention benefit can be

explained by changes in intermediate variables and outcomes (e.g., nutritional and biochemical measurements). These analyses will be conducted using relative risk regression methods, with appropriate account of measurement error in the intermediate variable measurements, using data obtained in a reliability substudy. Nested case-control and case-cohort sampling procedures (see next subsection) will be used in most such analyses since stored materials used to determine immediate variable values will not be routinely analyzed for the entire CT cohort.

Simple graphical displays and standard statistical methods will be used to present biochemical, bone density, and nutritional results by treatment group, clinic, and time since randomization during the course of the CT. Similar displays will describe the frequency and severity of adverse effects.

Observational Study

The ability to estimate relative risks for the outcomes of interest reliably in the OS as a function of baseline characteristics (exposures, behaviors or biologic measurements), or as a function of changes in such characteristics between baseline and three years is dependent on the accurate measurement of the characteristics (and outcomes) under study, and the accurate ascertainment and proper accommodation of all pertinent confounding factors. Even measurement error that is nondifferential in the sense that it is unrelated to disease risk given the 'true' characteristic values, can severely attenuate or otherwise distort relative risk estimates. Since many of the characteristics to be ascertained in the OS (e.g., nutrient intakes, blood cholesterol) are subject to noteworthy measurement error, a stratified 1% random subsample of the OS women will have repeat baseline information and specimens obtained at between one and three months following their OS enrollment, and again at between one and three months following their three year clinic visit. This reliability subsample will provide information of the reproducibility of the measurements taken, and can be used, under classical measurement error assumptions, to correct relative risk estimates for non-differential error in predictor and confounding variables. The 1% reliability sample will be stratified on age, racial/ethnic group, and socioeconomic group. The size of the OS cohort, and the comprehensive set of measurements to be obtained will allow a particularly thorough accommodation of confounding, by means of individual matching, stratification or regression modeling.

Relative risk regression methods (e.g., Cox, 1972) will also provide the primary data analytic tool for the OS. These methods, which can be thought of as an extension of classical person-year methods that avoids the assumption of constant disease risk for a study subject across the follow-up period, allow flexible modeling of the risks associated with the characteristics under study, as well as flexible accommodation of potential confounding factors, by means of stratification, matching, or regression modeling. Though less well developed they can also accommodate the types of reliability sample alluded to above (e.g., Pepe et al., 1989, Espeland et al, 1989; Lin et al, 1992), in order to produce 'deattenuated' relative risk estimates. Finally, relative risk regression methods are also readily adapted to accommodate nested case-control (Liddell et al., 1977; Prentice and Breslow, 1978) and case-cohort (Prentice, 1986) sampling schemes.

Nested case-control sampling proceeds by selecting for each 'case' of a study outcome one or more 'control' women who have not developed the disease in question by the follow-up time at which the corresponding case was ascertained. Additional matching criteria in the OS will typically include baseline age, clinic, and date of enrollment, and depending on the analysis may also include racial/ethnic or socioeconomic group, or other factors. Nested case-control sampling provides the only practical approach to reducing the number of OS women whose blood specimens need be analyzed and processed, if the measurements of interest cannot be assumed to be stable over time. For example, certain of the antioxidant concentrations to be measured in blood specimens are known to substantially degrade over the course of a few months or years of storage, in which case the follow-up-time-matched aspect of the nested case-control approach is essential to valid relative risk estimation. For measurements that are stable over time, however, case-cohort sampling could provide an alternative that has some decided advantages. Case-cohort sampling involves the selection of a random, or a stratified random, sample of the cohort to serve as a comparison (control) group for the cases of all the outcomes under study.

Analyses that relate change in risk factors to disease risk have particular potential for gaining insight into disease mechanisms. For example, the OS will provide a valuable forum for addressing the issue of whether

or not the association between low blood cholesterol (e.g., <160 mg/dl) and excess non-cardiovascular mortality derives primarily from persons who have experienced major reductions in blood cholesterol over the preceding three years. In fact the OS is large enough that such analysis could be restricted to women with relatively low baseline blood cholesterol (e.g., lowest two quintiles) in order to avoid a complicated interpretation if the effect of interest happened to 'interact' with baseline cholesterol measurement. Furthermore the OS, by virtue of ascertaining a range on non-specific markers of debility or disease (e.g., serum albumin, hemoglobin; cancer biomarkers; baseline and follow-up disease prevalence by questionnaire and physical exam) may be able to examine whether the excess mortality associated with reduced blood cholesterol can be explained by the presence of recognized or latent disease. The careful accommodation of measurement error in predictor and confounding variables is particularly important in such risk-factor-change analyses.

Section 1-A3, Protocol Appendix 3 provides power calculations for OS analyses as a function of disease rate, exposure frequency, relative risk, follow-up duration and, importantly, as a function of subsample sizes corresponding to racial/ethnic, age, and other important OS subgroups.

Clinical Trial and Observational Study

Separate analyses in both the CT and OS will be conducted according to self-reported baseline prevalence of the clinical outcome being analyzed. In fact, whenever applicable, relative risk analyses based on randomized CT comparisons will be accompanied by corresponding OS relative risk analyses. The comparability of these analyses is enhanced by the common aspects of baseline data collection procedures and outcome determination procedures in the CT and OS. Estimated relative risk functions from the two sources will take suitable account of prior "exposure" histories and of measurement error in exposure assessment. As indicated earlier (3.2.) under circumstances in which careful analyses of this type lead to substantial agreement between CT and OS results, it may often be reasonable to extrapolate the relative risk results beyond those examined in the CT, using the OS.

8. Osteoporosis Substudy

In order to ensure standardization of equipment and procedures used in bone mineral density measurements (BMD), such measurements will be restricted to the CT and OS participants at three VCCs. Urine samples will also be collected from these women. Changes in bone densities from baseline to prescribed follow-up times in the CT will be examined in relation to each CT component. The ability of such BMD changes to explain the relation between CT treatments and fracture occurrence will also be examined. In the OS, changes in BMD between baseline and three years will be studied in relation to baseline measurements, and the impact of including BMD measurements and changes in analyses of the relationship between fracture and corresponding risk factors will be examined.

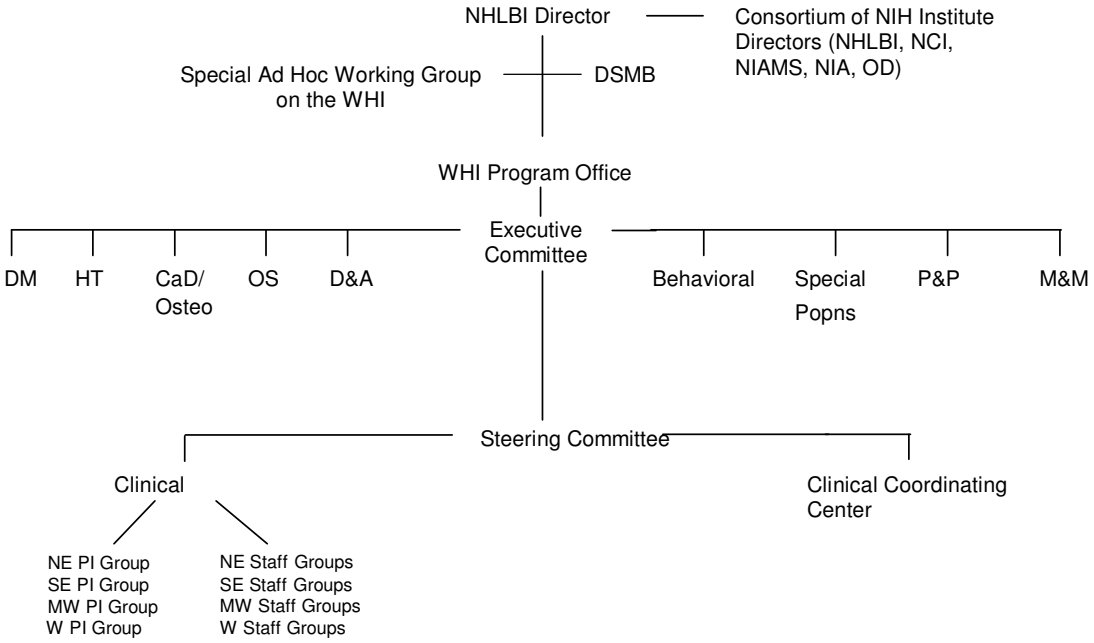
9. Ancillary Studies

Ancillary studies will involve CT or OS participants, and will involve the collection of data or specimens that are not part of the core study materials. Such studies may involve all or as few as one of the WHI CCs. Ancillary studies must not interfere with the basic objectives of the CT and OS. Proposed ancillary studies will have a separate protocol which will be reviewed in regard to impact on ongoing elements of the program, and for scientific merit, initially by the Design and Analysis Committee of the WHI Council, and following a favorable recommendation, approved by the NIH Project Office. Upon recommendation of the Design and Analysis Committee, ancillary studies of CT participants may also be submitted to the DSMB for approval. Separate informed consent must be obtained for each ancillary study, as must approval of the institutional review boards of the participating institution(s). External funding will typically be required. A separate policy document has been developed to govern ancillary study development (see *Section 3.4 - Ancillary Studies*).

10. Study Organization

The study organization includes the Program Office within the Office of the NHLBI Director, 40 Clinical Centers (CCs), the Clinical Coordinating Center (CCC) (including Core Laboratories and a Clinical Facilitation Center), and the various WHI study committees. The WHI Committees draw their membership from within the participating investigators and staff, and include a Steering Committee, an Executive Committee, and the various Advisory Committees of the Steering Committee. The CC-based committees are organized by region. Two external committees report directly to the NHLBI: the DSMB and the Special Ad Hoc Working Group on the WHI (SAHWG). An internal advisory committee is formed by the Directors of Consortium Member NIH Institutes. Some aspects of the study organization are shown in *Figure 4.*

Figure 4
Organization of the Study



Program Office

The study is being conducted out of the Office of the Director, NHLBI. NHLBI is the lead institute of a consortium of NIH institutes participating in the program. Within the NHLBI, the Director, WHI, is responsible for coordinating the program. The NHLBI Project Office oversees technical aspects of the program, and the Contracts Office oversees fiscal aspects.

Clinical Coordinating Center (CCC)

The CCC will: develop an initial and final Protocol and WHI Manuals, as well as other study materials in collaboration with other study units; train CC staff; collect and store data and biological specimens; analyze and report on data; monitor CC performance in collaboration with the project office via the Performance Monitoring Committee (PMC); establish and maintain the central laboratories, drug distribution center and specimen storage facility; arrange and coordinate committee meetings; and, develop a data collection,

management, analysis and reporting system, with quality assurance and quality control procedures and develop and maintain the WHI network.

Clinical Centers (CC)

Clinical Centers will: recruit women according to protocol inclusion and exclusion criteria toward satisfying the overall sample size requirements of the CT and OS studies. They further will perform baseline and follow-up activities in the CT and OS; provide study medications according to randomization assignment; instruct participants in medication use and procedure follow-up; ascertain clinical outcomes; collect and process biological samples; and perform study procedures according to protocol. WHI clinics will deliver the CCC-trained dietary intervention to participants, and will adhere to the dietary modification component of the CT according to the Protocol and WHI Manuals.

The CCs will collect participant data, accumulate and maintain participant files in a secure fashion, use the CCC-developed study database to enter and manage all participant data collected locally and perform local quality assurance measures. The CCC will be responsible for electronic data transmission and consolidation.

The CCs will participate in interim and final reports on all phases and activities of the program.

Special Ad Hoc Working Group on the WHI (SAHWG)

This committee is appointed by the Director, NIH to provide liaison with groups having special expertise or interest in women's health, and with the community-at-large. It advises the Office of Research on Women's Health (ORWH) Advisory Committee on Research on Women's Health (ACRWH) on strategies to promote the acceptance of WHI by women, helps identify potential problem areas which might have a negative impact on WHI, and participates in activities which promote the WHI. Its membership is drawn from the original Women's Health Initiative Program Advisory Committee (WHIPAC) which was comprised of public, lay, and scientific leaders. This SAHWG meets twice a year and will precede the ACRWH. A written report will be made to the ACRWH at their bi-annual meetings by the SAHWG representative.

Data and Safety Monitoring Board

This is an independent board appointed by the Director, NIH to monitor study progress, outcomes, and participant safety and to make recommendations in regard to protocol changes. The DSMB approves the procedures used to monitor the study for consideration of early stoppage of any of its components, and will make corresponding recommendations, when appropriate, based on the regular review of all pertinent study data, including adverse effects and unblinded outcome data in the CT. The CCC will provide study data for review by the DSMB. The DSMB reports its recommendations to the Director, NIH. The DSMB will normally meet twice a year.

Steering Committee

The Steering Committee provides overall scientific direction to the WHI. It is the arbiter of issues referred to it by the Executive Committee. It is empowered to make protocol changes, subject to confirmation by the Project Office. The Steering Committee may refer management and operational issues to the Executive Committee. The Steering Committee Members are WHI PIs (40 CC, 3 CCC) and a representative from the Program Office. The Steering Committee chair will be selected from its members, and will be the current chair of the Executive Committee. The Steering Committee will normally meet twice a year.

Executive Committee

The Executive Committee is a subset of the Steering Committee. The members of this committee work with the advisory committees to insure that issues and ideas are developed to a mature form prior to review and action by the Steering Committee. This committee also keeps an eye on the progress of the trials and the OS,

bringing important issues to the attention of the Steering Committeee. They will routinely meet monthly by conference call and face-to-face twice a year in conjunction with the Steering Committee meetings.

Advisory Committees

There will be 9 standing Advisory Committees of the Steering Committee. They will be the Dietary Modification, the Hormone Trials, the Calcium/Vitamin D and Osteoporosis, the Observational Study, the Special Populations, the Behavioral, the Publications and Presentations, the Design and Analysis, and the Morbidity and Mortality Committees. These committees are charged with advising the Steering and Executive Committees on protocol and policy issues pertaining to their respective areas of expertise. Each Committee will have a membership of 6-8, including the chair.

Regional Clinical Center Principal Investigator Groups: There will be 4 Regional CC PI Groups of about 10 members each. The Groups will consider any issues relevant to CC participation in the WHI CT/OS. The 4 regions will be: Northeast, Southeast, Midwest, and West.

Clinical Center Staff Groups: Clinical Center staff groups will be comprised of members of the 5 staff groups: Clinic Managers, Clinic Practitioners,Lead Nutritionists, Data Coordinators, and Outcomes Coordinators. Each staff group will consider issues in their respective areas of responsibility which are relevant to CC implementation of the WHI CT/OS, will identify problem areas, and exchange ideas for the solution of problems. Issues that have potential study wide implications or may result in changes to the WHI Manuals will be referred to the relevant regional PI group, which may bring the issues to the attention of the Executive Committee. A liaison member from the CCC will be designated for each of the regional staff groups.

11. Timetable

Phase 1 -	Clinical Coordinating Center and Vanguard Clinical Centers (16)		
Phase 1A -	Protocol Development	09/30/92 - 05/31/93	(8 months)
Phase 1B -	Training	06/01/93 - 08/31/93	(3 months)
Phase 1C -	Vanguard Recruitment and Follow-Up	09/01/93 - 01/31/95	(17 months)
Phase 2 -	Additional Clinical Centers (24)		
Phase 2A -	Training	09/30/94 - 01/31/95	(4 months)
Phase 2B -	Recruitment and Follow-Up	02/01/95 - 08/31/96 02/01/95 - 01/31/98	(19 months - VCCs) (3 years - Other CCs)
Phase 2C -	Follow-Up and Close-out 2D Visits*	09/01/96 - 03/31/05 02/01/98 - 03/31/05	(8 years, 7 months - VCCs) (7 years, 2 months - Other CCs)
Phase 2E -	Close-out Data	04/01/05 - 09/14/05	(5 months, 2 weeks)
Phase 2F -	Data Analysis	09/15/05 - 09/29/07	(2 years, 2 weeks)

*Assuming that recruitment is uniform over the designated three-year periods and that closeout visits occur on average three months prior to the end of Phase 2D, the average follow-up period will be 10 years in the VCCs and eight years, seven months in the other CCs for an overall average of 8.9 years, exclusive of the additional five years of follow-up for mortality and for breast and endometrial cancer incidence.

Section 1
Protocol for Clinical Trial and Observational Study Components

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Appendices

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